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PRIMARY ALDOSTERONISM

DEPARTMENT OF MEDICINE — SYMPOSIUM

RAYMOND MELLINGER, M.D., Moderator*
BERNARD THERIAN, M.D.** JOHN DITZLER, M.D.****
IRENE T. KLINE, Ph.D.*** ROGER SMITH, M.D.†
GERALD FINE, M.D.††

In the past two years, we have witnessed the brilliant exposition by Dr. Jerome Conn at Ann Arbor of a new clinical entity, primary aldosteronism. As we have learned the details of this syndrome, we have all watched for the first case at the Henry Ford Hospital and wondered when it might be found. Frequently, when such an intensive search is made, the case you seek is readily at hand, but has been overlooked. The patient to be presented this morning has been under our care for many years, but required the perceptive observation of new eyes in order to be truly seen. Diagnosis of this patient as a case of primary aldosteronism was made by Dr. Therian who will now tell us how he went about it.

Dr. Therian: E.D., a 10 year old Negro boy, was first seen 5 years ago in the Pediatric Clinic for the treatment of an upper respiratory infection. At that time he also complained of four other conditions which have continued until the present, namely, headache, polydipsia, polyuria and enuresis. The patient had been complaining intermittently of headache ever since he began to talk at the age of 3. The headaches were apparently severe, but information as to the mode of onset, the site or any associated symptoms was not clear. Polydipsia was also very severe with the child waking 3 or 4 times each night to drink 2 to 3 glasses of water each time. By day, he drank 2 to 3 quarts of milk in addition to many glasses of water. Polyuria was proportional to the polydipsia and enuresis was still present during the current hospitalization at the age of 10 years.

It was revealed in the past medical history that the child had 3 convulsive episodes at age 1 and 1½, all of which were associated with high fever. The family history was not considered significant in that both parents and 3 siblings were alive and well. There was no instance of arterial hypertension in the family members.

When first examined in the Pediatric Clinic in 1952, the child was considered to be somewhat retarded and was 12 pounds underweight for his height and age. There was a Grade I basal systolic heart murmur. The blood pressure was 230/140 and there was spasm of the retinal arterioles. The remainder of the examination was considered normal.

The patient was admitted to the hospital for evaluation of the severe hypertension. His blood pressure ranged from 160 to 200 millimeters of mercury systolic and 115 to 140 millimeters of mercury diastolic with one reading of 300/190 recorded.

*Division of Endocrinology
**Department of Pediatrics
***Endocrine Research Laboratory
****Division of Anesthesiology
†Department of Surgery
††Department of Laboratories

Three Regitine® tests and one Benzodioxane® test gave negative responses. The presence of strong femoral pulses and consistently elevated blood pressure in the legs ruled out the possibility of coarctation of the aorta. There was no evidence of glomerulonephritis, since repeated urinalysis failed to demonstrate albuminuria, hematuria or pyuria. However, the presence of polyuria was established during the hospital stay and the specific gravity of the urine varied from 1.004 to 1.006. The failure of the polyuria and hyposthenuria to respond to Pitressin administration excluded the possibility of diabetes insipidus due to antidiuretic hormone deficiency. There was no clinical, radiologic or electroencephalographic evidence of an intracranial lesion. At no time was there glycosuria and the oral glucose tolerance was quite normal. Chest x-ray demonstrated left ventricular hypertrophy, but an electrocardiogram was not done. The total direct eosinophil count was 272 with a 66% fall 4 hours after the intramuscular administration of 25 mg. of aqueous ACTH.

On the basis of the hypertension, the history of convulsive seizures, some mental retardation and the history of episodic perspiration, the diagnosis of diencephalic epilepsy was considered. Several electroencephalograms failed to demonstrate any compatible abnormality, however, and the subsequent course of the disease, including the response to anticonvulsive medication, failed to support this diagnosis.

In May of 1953, the patient was readmitted to the hospital with increasingly severe symptoms of headache, polydipsia, polyuria and enuresis. Repetition of the previous studies failed to demonstrate the cause of the disorder. Therapy with hexamethonium was initiated with some improvement in the number of headaches, but no significant reduction in the blood pressure.

In December 1955, the patient was admitted to the hospital for treatment of an upper respiratory infection. Following this, tonsillectomy and adenoidectomy were accomplished without incident. There had been no significant change in the patient's primary condition. Treatment with hydralozine and reserpine was initiated and continued until the present hospitalization. Although this treatment seemed to improve the patient's clinical course, his blood pressure remained at very high levels. On two occasions, he was admitted to the emergency room because of headache, hypertension and vomiting with a question of hypertensive encephalopathy or cerebral vascular accident. These episodes were transient and left no sequelae.

The present hospital admission began on July 21, 1957. In addition to the usual complaints of headache, polyuria, polydipsia and enuresis, the patient had experienced generalized myalgia although there had been no muscle weakness. The blood pressure varied from 160/100 to 240/180. Clinical examination revealed cardiomegaly, but no evidence of heart failure. The Grade I systolic murmur and the spasm of the retinal arterioles was again observed. The patient was clearly undersized and underweight for his age, but in other respects was clinically normal.

Cardiovascular, renal and endocrine function were again evaluated and led us to the diagnosis of primary aldosteronism. The first point in this diagnosis was the persistent, severe arterial hypertension which had been observed since the patient was 5 years old and, judged by the history of headache, must have been present since very early life. The second important fact was the persistent polydipsia, polyuria and hyposthenuria. Never in five years of observation had the specific gravity of the

urine been recorded higher than 1.007 except during a concentration test, when the highest obtainable specific gravity was 1.009. There was no observable response to Pitressin, which was administered on 2 different occasions. The third point in the diagnosis was the alteration of electrolyte metabolism. The pH of the urine had been persistently alkaline throughout the 5 years of observation. The serum potassium level was uniformly low, varying from 2 to 3 meq/L. The serum sodium was normal. Blood pH was elevated to 7.47 and the serum carbon dioxide averaged 28 meq/L. The determination of urinary sodium and potassium has given somewhat variable results, but there is a clear tendency to a high urinary potassium and low urinary sodium, in the face of the persistent low serum potassium. The fourth point in the diagnosis was the finding of a markedly elevated level of aldosterone in the urine. The assay performed in Dr. Conn's laboratory indicated a level of 150 micrograms in 24 hours. The excretion of 17 ketosteroids and 17 hydroxycorticoids was also determined. The 17 ketosteroids averaged 6.5 mg. for 24 hours and 17 hydroxycorticoids, 22.4 mg. for 24 hours, both of which values are considered above normal for the patient's age and weight. The patient responded sensitively to ACTH with a decided rise in the levels of both steroids.

In summary, our patient filled all the necessary criteria outlined by Dr. Conn for the diagnosis of primary aldosteronism. At no time had our patient demonstrated periodic weakness or paralysis or the intermittent tetany that has characterized other patients with the disorder, but occurrence of such episodes is not essential to the diagnosis. The evidence of generalized increase in adrenocortical hyperfunction is apparently unique to our case and will be investigated further. At no time were the clinical findings suggestive of adrenal virilism or Cushing's disease.

Adrenal exploration was undertaken after the administration of 100 mg. of cortisone intramuscularly. Both adrenal glands were found to be moderately generally enlarged, having a combined weight of 17 grams. Bilateral total adrenalectomy was accomplished and during the week since surgery, there have been no complications whatsoever. The blood pressure has fallen progressively to the present level of 150/100, and although there has been little change in the serum electrolytes or urine volume, it is anticipated that these will be corrected in a short time.

Dr. Conn has stated that of the known cases of primary aldosteronism, which now number over 50 patients, 5 or 6 have had their onset prior to the age of 20. All of these latter cases were found to have bilateral adrenocortical hyperplasia whereas the remaining cases were demonstrated to have adrenocortical tumors.

Dr. Mellinger: I want to point out that the diagnosis in this patient was established by readily available clinical criteria. The diagnosis was by no means dependent upon the demonstration of increased levels of aldosterone in the urine. On the basis of the history and the demonstrated abnormality in water and electrolyte balance alone, adrenal exploration was recommended. Although it is a fine thing to have our endocrine laboratory to perform special steroid studies, it is clear that this case could be diagnosed by quite ordinary means.

In order to give us some understanding of the mechanisms of this disease, we have asked Dr. Kline to discuss this hormone, aldosterone, with regard to its biochemistry, biosynthesis and mechanism of action.

Dr. Kline: It was recognized in 1934 that adrenal extracts from which all known steroids had been removed still contained an uncrystallizable, amorphous fraction which had a high potency for maintenance of adrenalectomized animals. Twenty years of experience with the chemistry of other steroids were required before a crystalline component of this fraction, the compound now known as aldosterone, was isolated and characterized chemically. Only during the past four years has this compound been available in even minute amounts, isolated from natural sources at great expense. No practical method of large-scale synthesis has yet been devised.

To make the chemical structure of aldosterone easy to remember and to relate its biological activity to structural modifications, I will show a few slides which indicate its similarities to and differences from the best known of the adrenocortical steroids.

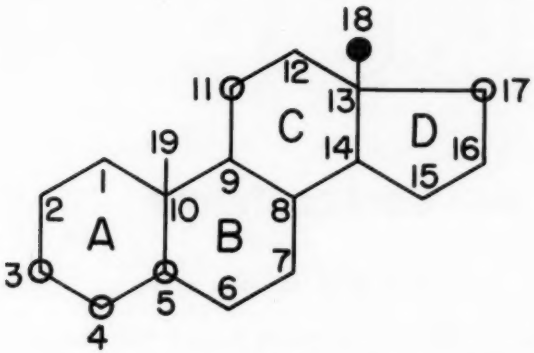
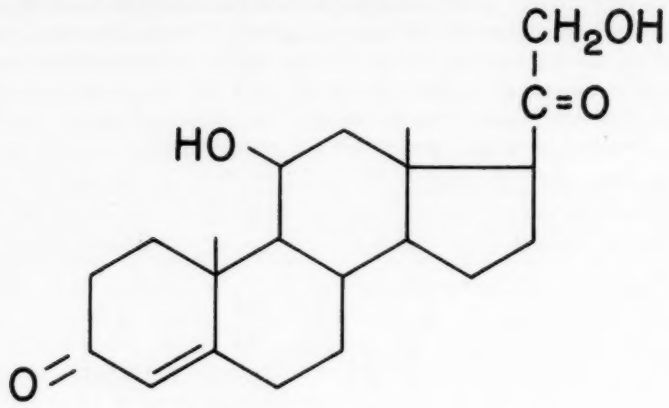


Figure 1
PARENT HYDROCARBON OF STEROID HORMONES

The first slide (Fig. 1) shows the basic ring structure, or the hypothetical parent hydrocarbon of all steroid hormones, a diagram familiar to all. It is a completely saturated hydrocarbon consisting of four rings lettered A, B, C and D. This conventional type of diagram indicates that all the valences of carbon not satisfied by linkage to adjacent carbon atoms are taken up by hydrogen atoms, even though the hydrogen is not shown. At carbon 1, for example, there would be understood to be two hydrogen atoms; at carbon 5, one hydrogen atom; at carbon 10, no hydrogen atom; and at carbons 18 and 19, three hydrogen atoms. The numbering of the carbon atoms, as indicated on the diagram, is from 1 through 10 counterclockwise around Rings A and B, clockwise around C and, finally, counterclockwise around Ring D to carbon 17. The carbon atoms numbered 18 and 19 are called angular methyl groups, and are numbered from right to left.

Carbon atoms indicated by open circles are the ones that are modified in one or more of the more familiar of biologically active cortical hormones. These modifications endow the different molecules with various biological activities and potencies. Carbon 18, indicated by a solid circle, is the position at which aldosterone alone among the known naturally occurring steroids hormones is uniquely substituted. This discussion will deal primarily with modifications of the parent hydrocarbon at the positions

indicated in Rings C and D. All biologically active adrenal cortical steroids are alike in Rings A and B, as illustrated in the next slide.



Corticosterone

Figure 2

A TYPICAL ADRENAL STEROID

Slide 2 (Fig. 2): Here is diagrammed the molecular structure of corticosterone, a typical corticosteroid, from which name are derived the common names of the rest of the more familiar adrenal corticoids. Note that it differs from the parent hydrocarbon at all of the five carbons which were indicated on the first slide by open circles. In Rings A and B, there is an Alpha, Beta-unsaturated ketone. That is, a ketone group replaces the two hydrogens on carbon 3 and a double bond forms between carbon 4 (Alpha-carbon) and carbon 5 (Beta-carbon) by the removal of one hydrogen from each of these carbons. This structural modification occurs in all active corticosteroids, as will be seen in subsequent slides. Corticosterone is also typical in having a 2-carbon side chain in place of one hydrogen atom at carbon 17. The carbons of this side chain both have oxygen functions, carbon 21 bearing an hydroxyl group and carbon 20, a ketone. Corticosterone is a fairly potent glucocorticoid, although not potent enough to be a valuable therapeutic agent. The substituent of the molecule which specially endows it with glucocorticoid activity is the oxygen function, present as an hydroxyl group, at carbon 11. All compounds with distinct glucocorticoid activity have an oxygen group at C_{11}, either as an hydroxyl or a ketone.

Slide 3 (Fig. 3): Glucocorticoid activity is intensified if in addition to an oxygen at C_{11} an hydroxyl group replaces the remaining hydrogen at C_{17}, as shown in the structure for Compound F, cortisol or 17-hydroxycorticosterone, which is identical otherwise to corticosterone. Cortisone (Compound E, 11-dehydro, 17-hydroxycorticosterone), more potent than corticosterone but less potent than cortisol, differs from cortisol only in that the oxygen at C_{11} is present as a ketone rather than as an hydroxyl

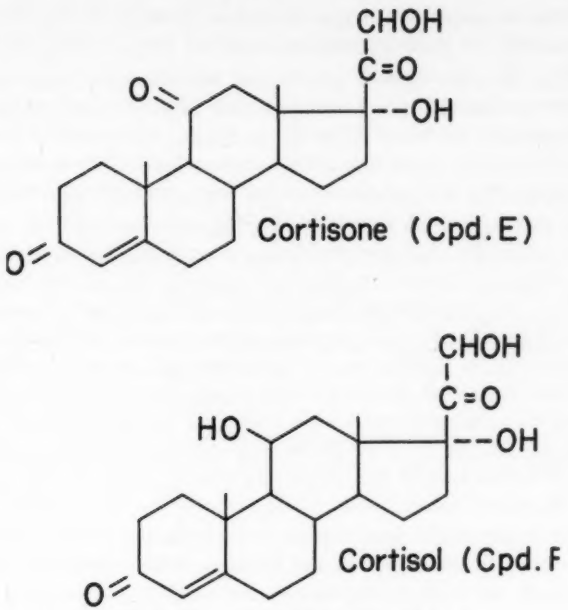
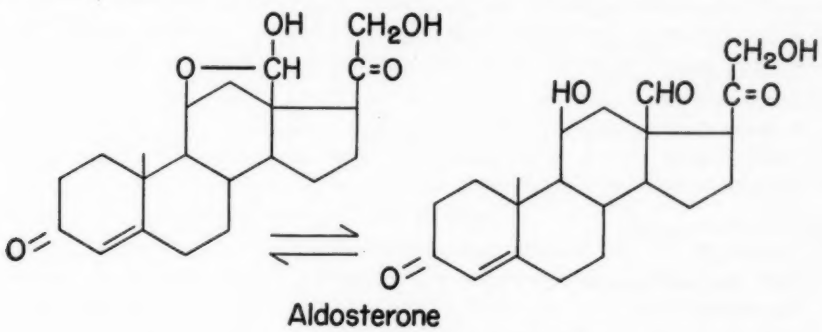


Figure 3 GLUCOCORTICOIDS



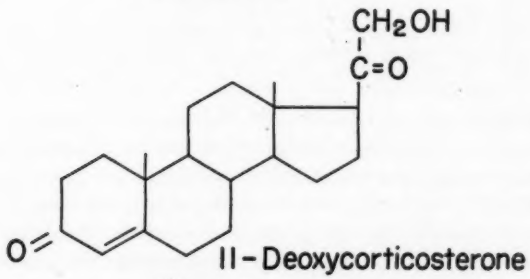


Figure 4 MINERALOCORTICOIDS

group. Small differences in the oxygen functions in Rings C and D, then, can be seen to be responsible for great differences in potency of glucocorticoids.

Slide 4 (Fig. 4): Shown here are the two most potent mineralocorticoids, aldosterone and desoxycorticosterone. Looking first at desoxycorticosterone, note that its name indicates absence of the 11-hydroxyl group of corticosterone. Since it is also without an hydroxyl at C_{17}, we can predict from what we know of the relation of structure to function that this compound is practically devoid of glucocorticoid activity.

With the preceding analysis of corticosteroid structure in mind, let us now examine the aldosterone molecule. Aldosterone in crystalline form has an aldehyde group at C_{18}, hence its name. In solution it exists as an equilibrium of two molecular species as shown here, the hemiacetal being formed between the C_{18} aldehyde and C_{11} hydroxyl groups. Referring specifically to the crystalline aldehyde form for the sake of simplicity, note that aldosterone is identical to corticosterone except for modification at one additional carbon atom. One would therefore expect it to have glucocorticoid activity equal to that of corticosterone and in this respect to be different from desoxycorticosterone. Its unique modification, the aldehyde group at C_{18}, must be assumed to be the structural feature which endows this compound with its extremely high potency in the regulation of electrolyte metabolism. Its activity in electrolyte regulation has been known since its discovery, because one or more compounds with such activity were predictable on the basis of the activity of the amorphous fraction of adrenal cortical extract and because its isolation was based on assays for such activity.

*TABLE 1.: BIOLOGICAL ACTIVITIES OF CRYSTALLINE ALDOSTERONE

Tests	Potency of Aldosterone Relative to:	
	Desoxycorticosterone	Cortisone
For mineralocorticoid activity:		
Sodium Retention	25 x	—
Potassium Excretion	5 x	—
Na/K Excretion	120 x	—
Maintenance Adx. Dog	25-30 x	—
For glucocorticoid activity:		
Cold Stress	>	= or >
Liver Glycogen Deposition	30 x	1/3
Eosinophil Depletion	—	1/2 — 1/4
ACTH Suppression	—	1/2 — 1/3

*Modified from Gaunt, R., "Biological Studies with Aldosterone (Electrocortin)" in "The Human Adrenal Cortex", Ciba Foundation Colloquia Vol. VIII, edited by G. E. W. Wolstenholme and M. P. Cameron and published by Little, Brown and Co., Boston, 1955. Table reproduced by permission of author and publisher.

On the last slide are comparisons of the biological activities of aldosterone, desoxycorticosterone and cortisone (Table 1). This table is reproduced from a paper by Gaunt, presented at a Ciba Foundation Colloquium and published in the volume entitled, "The Human Adrenal Cortex". From the data it can be seen that aldosterone does have the glucocorticoid activity predictable from its possession of an hydroxyl group at C_{11}. Its potency in regulating electrolyte metabolism is shown by each of the four pertinent tests to be greater than that of desoxycorticosterone. The relative

potencies of these two compounds are not the same in the four tests, however, indicating subtle differences between them in this physiological activity.

We have been accustomed to categorizing adrenal steroids as either mineralocorticoids or glucocorticoids. This has been convenient but not entirely correct since each compound, even though predominantly of one activity, also possesses the other to a minor degree. Aldosterone forces us to recognize this point because it combines not minor but moderate glucocorticoid activity with the highest mineralocorticoid potency.

Dr. Mellinger: The glucocorticoid activity of aldosterone certainly has biochemical and physiologic significance, although the amount of the hormone secreted by the normal adrenal is so minute that its glucocorticoid or life-maintaining property would be negligible. However, this is the naturally occurring adrenal cortical hormone with the most significant ability to regulate body sodium and potassium and their coincident effects on acid-base and water balance.

Now that we know something of the biochemistry and physiology of these adrenal substances, we will ask Dr. Fine to demonstrate the adrenal histology of the patient presented.

Dr. Fine: Both adrenal glands in this patient were similar and both showed an irregular yellow thickening of the cortex. Microscopic examination revealed that this nodular hyperplasia is confined to the zona fasciculata or the middle zone of the adrenal cortex. The zona glomerulosa is so thin it is difficult to delineate. High power magnification of the cells of the hyperplastic zones shows a marked pleomorphism of the nuclei. There was no discrete tumor or adenoma, the pathological diagnosis being adrenal cortical hyperplasia. We could not distinguish the adrenal glands in this case from those of patients with Cushing's syndrome due to adrenal cortical hyperplasia.

Dr. Mellinger: Thus, we have presented the first case of primary aldosteronism at the Henry Ford Hospital — diagnosed clinically, established biochemically, and confirmed pathologically. This is believed to be only the sixth or seventh case of diagnosed primary aldosteronism due to adrenal cortical hyperplasia. An adrenal cortical tumor will be found in almost all cases with this syndrome.

Dr. Ditzler administered the anesthetic to our patient at the time of surgery. He encountered problems which are worthy of our attention so I have asked him to discuss them here today.

Dr. Ditzler: Although it is true that this child's surgical course was ultimately quite uneventful, this was not accomplished without depositing some new plaques on the coronary arteries of the anesthetist. Some time ago we discussed with this group the complications we have encountered administering general anesthesia to patients receiving rauwolfia derivatives. This child is a striking example of these complications. Preoperatively his blood pressure averaged about 200/140. He received minimal premedication and arrived in the operating room awake, alert, and cooperative, able to talk with us at great length about what we were to do. His blood pressure, to our surprise, was 110/70. When we turned him to a right lateral position the pressure declined to 80/40 and when we turned him to a left lateral position it fell to 50/0. This observation was repeated several times during the following hour, and because

of the hypotension the anesthetic was not administered. The operation was postponed for one week during which time no rauwolfia derivatives were administered. Although we prefer an interval of three weeks without such medication before undertaking elective surgery, the operation was rescheduled after the patient had received no rauwolfia for two weeks. He received the same premedication and the blood pressure on arrival at the operating room the second time was 150/100. Placing the patient in the lateral positions did not effect the blood pressure, but induction of anesthesia with a minimal dose thiopental (5 cc.) induced a fall in the blood pressure to 50/0. With the use of vasopresser drugs we were able to control the blood pressure and proceed with the adrenalectomy. We feel that these events do represent a synergism between barbiturates and the rauwolfia compounds which must be remembered in planning elective surgery.

Dr. Whitehouse: I would like to ask two questions. First of all, was a renal biopsy done at the time of surgery and second, was consideration given to the possibility of subtotal as opposed to total adrenalectomy?

Dr. Fine: Histologic examination of the tissue removed by the renal biopsy demonstrated no abnormality. The hydropic change of the renal tubules which has been described in other cases of primary aldosteronism was not present.
Dr. Mellinger: I would like Dr. Roger Smith of the surgical team to comment on the second question of Dr. Whitehouse.

Dr. Roger Smith: In adrenal surgery, as in thyroid surgery, it would be a fine thing to know exactly what part of the endocrine gland, if left in place, would secrete the optimum amount of hormone to maintain health. This is difficult in thyroid surgery and is even more difficult in adrenal surgery since our experience here is less extensive and the surgical problems involved are considerably greater from the technical standpoint. There has been some experience with subtotal adrenalectomy, but it is my impression that patients who have had this operation require maintenance doses of cortisone similar to those of completely adrenalectomized individuals. In addition, draining sinuses and wound infections have been encountered which may represent infarction of the remaining portion of the adrenal gland. Furthermore, one encounters the problem of possible recurrent hyperfunction of the remaining tissue. If we can use the thyroid surgery analogy once more, it can be observed that the hyperfunctioning remnant of thyroid tissue frequently hypertrophies after subtotal thyroidectomy with a recurrence of the thyrotoxicosis a few years after the operation. We do not know whether the hyperplastic adrenal gland of primary aldosteronism has the same potentialities, but it is certainly a logical possibility. A repetition of adrenal surgery is not nearly so easy as is the surgical removal of recurrent hyperplastic thyroid tissue. We prefer total adrenalectomy because of the difficulty in leaving a remnant of optimum size, because of technical surgical considerations, and because we wish to avoid any possibility of re-operation. We were strengthened in this decision by the belief that the patient would be in no better circumstances with a subtotal adrenalectomy than with the total procedure.

Dr. Mellinger: I believe that if we in the medical service had urged Drs. Smith and Szilagyi to undertake subtotal adrenalectomy we might have persuaded them in this

course. For the reasons that Dr. Smith has given we also preferred total adrenalectomy. The point should be emphasized that the reported cases in whom subtotal adrenalectomies have been done exist only by virtue of taking 25 milligrams of cortisone a day. In other words, these patients are clinically no different from patients who have been subtotally adrenalectomized.

Dr. Richmond Smith: It is my opinion that total adrenalectomy is preferred from yet one other view point. Even if the adrenal remnant is sufficient to maintain the patient's needs for a day-to-day existence, it very likely would prove inadequate to meet any severe physical stress. The patient who knows he is utterly dependent upon exogenous hormones for survival may prove to be more secure than the patient who does not have this sense of dependency in his daily life, but who in actuality remains a particularly vulnerable person.

Dr. Mellinger: Dr. Miller can bring us the experience of the Mayo Clinic where subtotal adrenalectomy has been done with great success.

Dr. Miller: Subtotal adrenalectomy has been a great success at the Mayo Clinic. Most of their patients with Cushing's disease undergo subtotal adrenalectomy and most of them are fully capable of normal existence without steroid supplements. I point this out, not in a critical sense, but only to show that subtotal adrenalectomy is a feasible procedure.

Dr. Mellinger: Of course, the situation may be different in Cushing's syndrome and in primary aldosteronism. To my knowledge, no patient who has undergone subtotal adrenalectomy for primary aldosteronism is able to live without supplemental glucocorticoids. In Cushing's disease there is presumably a direct stimulating hormone which will induce hyperplasia in the adrenocortical remnant. As far as we know, there is no trophic hormone that leads to the hyperplasia of the adrenal cells producing aldosterone. Control of these cells is not completely delineated, but the probable stimulus to secretion of aldosterone lies in body fluid compartments and electrolyte balance. There is no evidence that the pituitary or other endocrine organs play a significant direct role in the control of aldosterone or in the production of the hyperplasia which has been demonstrated today.

SOME UNUSUAL MEDICAL SYNDROMES*

ROBERT H. DURHAM, M.D.**

The word "syndrome" has been in recognized use since 1541. Even after more than 400 years it continues to be one of the most misused terms in our medical vocabulary. It is most frequently used as a synonym for such terms as symptom complex, clinical entity, pathologic process or disease.

Dictionary definitions of a syndrome are vague. The term is defined as a concurrence or running together (togetherness) of abnormal signs or symptoms. In the Spanish language it is interpreted as any disturbance of physiology. As used in French medical literature, this convenient and elastic term seems to be an indispensable one. It is rarely used in German medical writing. More correct usage has broadened the concept to include as a composite group two, three, or even four totally unrelated conditions which occur in combination sufficiently often to symbolize a syndrome. The gradual evolution and final recognition of the component parts of a typical syndrome may require years or decades. A characteristic example is Van der Hoeve's syndrome, which consists of blue sclerae, brittle bones and otosclerosis with deafness. Innumerable other odd combinations could be cited. Unfortunately, the literature also abounds in such loosely used terms as "brassiere strap syndrome".

Categorically, syndromes may be classified as (1) eponymic, those which bear the name of one or more individuals who contributed to the clarification of the subject, and (2) descriptive. Most true syndromes are either congenital, familial or the result of genetic abnormality. Frequently only a partial anomalism is present. Syndromes occur in various fields of medicine, especially in disturbances of multiglandular physiology, dermatology, neurology, and opthalmology. As a rule, they evoke more interest and are more challenging than a disease, because the relationships are obscure, and the etiology is usually unknown. Their variable characteristics will be more evident after a review of the small group herewith presented.

"Women Who Fall" syndrome. From this name it may be inferred, perhaps not incorrectly, that a permissible synonym is "fallen women." This is a narcissistic syndrome, occurring only in women and characterized by stumbling and falling without apparent reason. While talking or walking and without encountering the slightest obstacle, the patient may fall. After one or more such incidents, a neurologist is usually consulted. If the syndrome is promptly recognized, the patient may be referred to a psychiatrist or to a psychoanalyst. As an example, psychoanalytic study of one woman who suddenly fell to the street indicated that at the moment she fell she was thinking that the imminent betrayal of her husband was well justified because he was effeminate. An analytic interpretation of the emotional and personality make-up of such a patient indicates an ego that is weak, inconsistent, and, in fact, almost nonexistent. Under certain circumstances, this timid ego may be submerged by anxiety and wholly or partly obscured. When the obscuration is total and the ego vanishes for a fraction of a second, falling occurs.[1]

---

*Read before the Texas Club of Internists October 8-10, 1957. The subjects presented were selected from a forthcoming book to be published by Paul B. Hoeber, Inc., Medical Book Department of Harper & Brothers, New York.

**Physician-in-Charge, Division of General Medicine.

"Alice in Wonderland" syndrome. It requires an elastic imagination to categorize as a medical syndrome the story of "Alice in Wonderland", which has delighted so many readers. Through psychoanalytic interpretation, the illusionary dreams, feelings of levitation, and alteration in the sense of passage of time which Alice experienced have been made more understandable; in essence, Alice trod the paths and byways of a wonderland well known to her creator, Lewis Carroll. He suffered severely from migraine and many epileptic and migrainous patients experience bizarre disturbances of body image. Alice, as you will recall, dreamed that she was either remarkably tall or remarkably small, was a dual personality, and had illusions concerning the size, position, or distance of objects. In other words, according to the analysts, the record of her bizarre experiences was essentially a mirror of the experiences suffered by Carroll as a sequel to his migraine. According to neurophysiologists, the site of origin for such psychic alteration may perhaps be in the parietal lobe. Some degree of reproduction of similar symptoms has been reported to occur after electrical stimulation of this area.[2]

Bogorad's syndrome. This title is not a euphonious one. Its synonym, the syndrome of crocodile tears, evokes more interest. This latter title has been applied because it was formerly believed that the crocodile wept hypocritical tears while devouring its victims. Clinically, this syndrome is characterized by paroxysms of lacrimation which occur during eating. The exact etiology is obscure, but the condition has been observed after facial nerve palsies or in association with certain tics. The nerve fibers which normally cause salivary gland stimulation become misdirected to the lacrimal glands and produce tears. The condition is apparently due to a deviation of regenerating axons from their normal course. A similar overflow of nerve impulses into adjacent nerves has been seen in other neurologic conditions which will be mentioned.[3]

Marcus-Gunn syndrome. This neurologic phenomenon is also known as the jaw-winking syndrome. Characterized by conspicuous increase in the width of the eye-slits, the syndrome occurs during mastication. In some instances, there is a rhythmic elevation of the upper eyelid when the mouth is opened and a drooping of the lid as the mouth is closed. Widening of the palpebral fissures is not limited in its occurrence to chewing motions. It has also been observed during loud speaking or shouting, while abducting the jaw to one side, and in children during episodes of crying. The condition is congenital, often occurs in successive generations, and one report described eighteen affected members in three generations. Neurologists and neurophysiologists suggest that the condition is best explained by a supranuclear lesion, a probable connection of the third and fith cranial nuclei in the posterior longitudinal bundle or the corticopontine tract.

The two neurologic syndromes just described have their clinical counterpart in other synchronized neuromuscular motions. Observations show that cats raise their eyebrows as they open their mouths to eat. In addition, the writer has also observed and demonstrated in a posthemiplegic patient flexion of the affected hand and forearm and some elevation of the entire arm which invariably occurred during the act of yawning.

These several neurologic aberrations strongly suggest that under certain circumstances there may be a short-circuiting or misdirection of impulses from one

cranial nucleus or nerve into another with consequent disturbance of effects upon the end organs.[4]

Gilles de la Tourette syndrome. This interesting syndrome was first described in 1884 by a well known French neurologist, whose eponym it bears. It is characterized by incoordinated motor movements, including facial twitchings or tics, blinking of the eyes, or jerkings of the face, arms, or legs. These movements are often accompanied by verbal exclamations, which include profanities, swearing, and coprolalia, that is, the uttering of filthy words. The verbalization is often explosive, accompanied by imitative gestures. The onset of this strange behavior often occurs by the age of ten years, and the extent of the profane vocabulary at this age is sometimes astounding. The syndrome is not infrequently brought on by strong emotional stimuli or sudden noises. Affected patients find it difficult to concentrate and are easily distracted. With increasing age they tend to withdraw from social intercourse. The etiology is not known. Psychotherapy has been tried without noticeable success.[5]

Stiff-man syndrome. This peculiar myopathy is characterized by generalized muscular spasm, rigidity, and myalgia. The onset may be rapid, developing within a few days, or more slowly, extending over several months. There is marked fluctuation in the intensity of the symptoms; the severity of the pain parallels the degree of muscular spasm. Muscles of the trunk are usually first affected. As the disorder progresses, other muscle groups become involved symmetrically until the effect of the over-all rigidity resembles a poker spine. The gait becomes stiff and laborious, and the patient justly becomes apprehensive about impending total rigidity.

Detailed studies of numerous patients have revealed no biochemical disturbance, except reducing substances occasionally found in the urine. No etiologic concept has been formulated. It has been postulated that the rigidity may occur reflexively by way of the spinal cord or that the basal ganglia are involved. Antispastics have not been helpful in treatment; in fact, no measure has been effectual.[6]

Restless legs syndrome. Paresthesias and a sensation of restlessness felt in the legs, with inability to sit still, are characterstics of this syndrome. It occurs in mentally healthy patients, more often in women. Symptoms are felt only when the legs are at rest and consist of an unpleasant creeping sensation which seems deep in the tissues of the leg, more often below the knee, although the thigh is sometimes involved. The condition may occur while in the theater or at home, perhaps while entertaining certain friends. Many patients will admit that the symptoms are most likely to occur during a period of boredom. Movements of the legs or walking affords relief. Symptoms are usually periodic. In severe cases the feeling continues for several hours and may occur in rare instances night after night for years. The cause is not known, and no cure has been found.[7]

Kleine-Levin syndrome. This unusual condition is characterized by hypersomnia and abnormal hunger. It apparently belongs to the group of narcoleptic disorders. The hypersomnia occurs in crises and is accompanied by slight agitation or mental confusion. In one recorded case observed in Coventry, England, the patient slept like a child during an eleven-hour period of intensive bombing and destruction of the city. Attacks occur at irregular intervals and may last two or three days. Males are more frequently

affected. Various studies have thrown no light whatsoever on the cause. Electroencephalograms have for the most part been normal. There is no evidence of a frontal lobe tumor which often causes an excess of hunger. Glucose tolerance tests are also normal, and there is no evidence to suggest a pancreatic tumor. Some observers believe that the syndrome is the result of a lesion in the area of the hypothalamus and third ventricle.[8]

Anton's syndrome. This unusual eponymic syndrome was first described by Anton over half a century ago. Also known as amaurosis denial syndrome, it consists of blindness with confabulation and denial that the blindness exists. Besides stoutly denying their inability to see, afflicted patients describe objects which they believe they can see. Eventually, however, the patient may recognize that the blindness exists. The mechanism of the syndrome is not known, but it has been postulated that it is due to isolation of the diencephalon from the occipital lobe. In certain cases, the denial part of the syndrome is thought to relate to a lesion of the calcarine-thalamic connections.[9]

Spasmodic laughter syndrome. Episodic outbursts of irrepressible laughter which occur without any idea of merriment are occasionally observed. The laughter may be spontaneous without any apparent psychic stimulation, or the paroxysms may be initiated by such simple movements as pointing a finger at the unfortunate subject. The episodes are usually recurrent, may be relatively brief, or may continue incessantly to the point of complete neuromuscular fatigue.

Pathologic laughter is usually a sequel to some type of organic brain lesion and may occur in association with epilepsy, postencephalitic states, more rarely in multiple sclerosis, or pseudobulbar palsy. It also occurs after prefrontal lobotomy. Cerebral pathology may usually be demonstrated by electroencephalographic study.

A more severe type of almost continuous episodic laughter has been described as a part of the symptom complex of Kuru, a puzzling, highly-fatal, epidemic, neurologic syndrome which was recently discovered in New Guinea. Because of the associated lesions, the prognosis of the spasmodic laughter syndrome is usually grave.

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POTASSIUM — SOME PRACTICAL ASPECTS OF THE PHYSIOLOGY OF ITS ELECTROLYTE BALANCE

FRANCES E. NOE, M.D.*

There is a general tendency in the field of clinical medicine to reduce electrolyte replacement therapy to a few simple principles, the application of which is usually based on a minimum of routine laboratory procedures. Such practice is a matter of practical necessity, not of convenience or neglect, and one could not expect the majority of clinicians to be familiar with the maze of academic and often hypothetical material published in this field. However, there are noteworthy clinical applications which should be culled from the literature and added to one's stock of such knowledge from time to time.

A new entity, and by no means a homogeneous one, must be added to our concepts of electrolyte balance — the intracellular space. This has made it more important than ever to treat the patient, not the flame photometer results. We are more and more into an era which makes it possible by the application of a few fundamental principles to prevent the electrolyte imbalance and not have to treat it, as the pathophysiology of electrolyte disturbance becomes an essential part of general medical knowledge.

The phenomenon of potassium transfer to extracellular fluid with concurrent increased intracellular sodium content and relative acidity is by now familiar to most clinicians but what are the clinical implications of such an exchange? How do existing electrolyte disturbances such as chronic respiratory acidosis affect body response to newly imposed stress such as surgery? This paper will attempt to sketch briefly material from recent literature which may help to answer such questions.

POTASSIUM DEFICIENCY

The signs of cellular potassium deficiency as defined and described by means of experimental studies present a specific clinical syndrome complete with electrocardiographic changes. However, such a condition seen in the post-surgical patient or accompanying a refractory case of chronic congestive failure may not be readily apparent. Usually the first complaints of the patient with early potassium deficiency are weakness and lassitude. With severe hypokalemia muscular weakness may advance to a state of flaccid paralysis, usually of the lower extremities, but involvement of the respiratory muscles may mask the basic disturbance. Smooth muscle dysfunction results in gastric and intestinal distention, nausea and vomiting, and occasionally paralytic ileus. With prolonged deficiency renal functional changes may occur which are not easily reversed and McAllen and others have reported myocardial pathology including necrosis which may present the clinical picture of myocardial infarction.[1]

CELLULAR TRANSFER OF POTASSIUM

Potassium leaves the cells when metabolism ceases, and likely does so to a lesser extent when reversible metabolic disturbances such as anoxia or insulin deficiency occur. It has been postulated that the cellular cation deficit is replaced by sodium to the extent of 2/3 of the loss and by hydrogen ion for the remaining third, the hydrogen ion shift promoting a relative extracellular fluid alkalosis. Experimentally

*Division of Chest Disease, Research Resident.
Present address: Department of Medicine, Wayne State University College of Medicine, Detroit, Michigan.

and clinically it appears that the concomitant lowered sodium plasma level which may occur cannot be raised to normal levels when potassium deficiency prevents replacement of intracellular potassium. Though there are mechanisms for the retention of sodium when depletion of that ion is threatened, there seems to be little evidence for significant support of cellular stores of potassium and Berliner and others have shown that even in dire deficiency states experimental animals and humans may continue to lose large amounts of that ion in the urine.[2]

EFFECT OF pH

An increase in plasma pH tends to enhance movement of potassium into the cells; acidosis or decreased pH promotes potassium transfer from cells to extracellular fluid. In potassium-depleted animals the same relationship between serum potassium and pH is evident but serum levels tend to be somewhat lower. However, any significant rise in serum potassium is accompanied by transfer of potassium into the cells and the opposite transfer occurs with serum level decrease. Darrow develops the clinical implications of these experimental findings as follows.[3]

In alkalotic states one would expect the serum potassium to be low even if cellular stores are normal, and especially low if cellular deficiency is present. Serum levels in alkalosis are not likely to be high unless renal function is impaired or unless disturbances of cellular metabolism cause intracellular loss. The increased tendency for potassium transfer into the cells when the pH of extracellular fluid is high has a bearing on therapeutic measures in alkalotic states with potassium depletion. Sudden decrease in the serum potassium level may accompany potassium therapy due to intracellular transfer when cellular stores are depleted, much as insulin may bring on low serum levels symptoms in the diabetic patient.

In acidosis the intracellular transfer of potassium is retarded by the decreased pH and serum levels are likely to be normal or high in spite of considerable cellular deficit. Low serum levels in acidotic states usually indicate severe deficits of cellular potassium. However, renal functional impairment or significant changes in cellular metabolism must be considered here also. It must be remembered too that sodium bicarbonate used to correct acidotic imbalance will tend to accelerate cellular transfer of potassium and may cause rapid changes in serum potassium levels in spite of acidosis.

ACIDOSIS

Acidosis with potassium deficiency is not an uncommon clinical association. Many conditions leading to acidosis also result in potassium deficit; the most frequently seen are probably severe diarrheal states, starvation and base-depleting forms of renal impairment. More often, however, potassium deficiency is superimposed on an unrelated acidosis or conversely, the patient with chronic potassium deficiency becomes acidotic.

Bland states that though moderate potassium depletion in itself does not cause severe symptoms, it may and usually does do so when associated with metabolic acidosis. The potassium depletion of alkalosis does not result in nearly so ill a patient as the same degree of depletion in the presence of metabolic acidosis. Muscular paralysis occurs more readily and earlier in the latter instance also.[4]

Respiratory acidosis in itself does not seem to promote disturbances of potassium balance. The kidney compensates for the increased CO_2 tension of the blood by retaining bicarbonate and sodium ion and excreting hydrogen and chloride ions. However, it is most important to differentiate between respiratory acidosis and metabolic alkalosis. Both are characterized by a high serum CO_2 content and low serum chloride and the urine in both states may be acid in reaction and contain increased amounts of ammonia and chloride. Usually the clinical history and physical examination are sufficient to allow diagnosis; if not, a pH determination is the only reliable test unless measurement of CO_2 tension is available. The pH of the urine may help — an alkaline pH especially if associated with shallow respiration in the absence of pulmonary disease suggests metabolic alkalosis.[4]

Bland notes that potassium depletion may be associated with an over-all acidosis of body fluids even in the presence of a raised serum bicarbonate. As the result of such findings he suggests treating clinical potassium depletion with potassium in combination with anions, such as citrate or lactate which can be oxidized by the body.

In metabolic acidosis the renal demand for base for acid excretion depletes potassium as well as sodium. In addition the compensatory excretion of bicarbonate requires cations and accounts for more potassium loss. In the process of repair, experimental work reveals that the low bicarbonate level due to acidosis will not return to normal level if deficits of sodium and potassium have developed, since renal excretion of hydrogen ion and retention of bicarbonate can take place only if an adequate supply of those cations is present.[3]

Sodium replacement in acidosis cannot be estimated from the bicarbonate level, as has been assumed in the past. Cellular cation deficit should be replaced by potassium. Some of the replaced sodium will enter the cells due to the increased plasma level but the extent of such transfer of sodium is not predictable. Therefore, Darrow recommends fluid replacement in acidosis consist of solutions resembling extracellular fluid, such as Hartmann's Ringer lactate. He suggests potassium in sufficient amount to cover daily loss as well as some of the deficit be started simultaneously or as soon as restitution of renal function and cellular metabolism are adequate.

It is worth remembering that intestinal losses as well as renal losses can be a major factor in potassium depletion. Colonic loss of potassium as high as 70 mEq. per liter of stool has been reported in severe diarrhea.[5] Schwartz and Relman have described severe potassium deficiency resulting from excessive use of cathartics over long periods of time with no apparent alkalosis.[6] Decreased intake, dehydration and rapid therapeutic infusion of sodium solutions often contribute to the potassium deficit of diarrheal illness.

Potassiam replacement therapy in diabetic acidosis has been generally accepted. If adequate potassium is available, the not uncommon practice of overcompensating for sodium loss by lactate or bicarbonate administration need not result in serious potassium deficit; however, there is still danger of making the patient hypernatremic.

The mechanism of the drop in potassium and phosphate stores in developing diabetic acidosis has not been clearly defined. That it is intimately connected with

cellular balance and the impaired carbohydrate oxidation seems evident from the rapid sequence occurring with therapy but the serum levels do not reflect the changes, according to Bland, and may even rise as renal function declines with increasing acidosis. Therefore, with very severe acidosis the serum levels are frequently high rather than low.

ALKALOSIS

A great deal of clinical and experimental evidence indicates that metabolic alkalosis will not develop if potassium intake is sufficient and that it cannot be corrected if potassium deficit exists. The renal compensation for metabolic alkalosis is essentially retention of hydrogen ions. This is accomplished by excretion of bicarbonate or organic acids with sodium and potassium. Since, as Darrow points out, there is usually a relative or absolute excess of sodium in the body in alkalosis, renal compensation is likely to produce only deficits of potassium.

Apparently potassium excretion is necessary to prevent alkalosis and to maintain plasma chloride levels. Either potassium chloride or potassium bicarbonate will bring about normal balance in potassium deficiency associated with alkalosis. Sodium from the cells is excreted with bicarbonate or with chloride if an excess of either ion is present.

Medical conditions prone to develop metabolic alkalosis include those tending to potassium deficiency. Vomiting, diarrhea, or continuous tube aspiration and prolonged administration of ACTH or cortisone-like substances are probably the most commonly encountered in medical practice. Excessive use of alkaline preparations, diuretic agents or the impaired hormonal response in certain renal or cardiac edematous states may be etiological factors.

Chronic respiratory alkalosis has seldom been the subject of physiological study. Hyperventilation experiments of relatively short duration have revealed no sustained impairment of sodium or potassium balance secondary to pH or pCO_2 changes but marked increase in excretion of base, particularly potassium, and retention of phosphorus have been noted.[7] Recent studies with respirator patients who have been over-ventilated indicate that renal compensation in such cases may not be complete for as long as 5 months.[8] In general, evidence seems to indicate that potassium deficit should be suspected in patients with central nervous system disease or psychic disturbances causing hyperventilation for long periods of time.

Acute respiratory alkalosis is seldom a problem for treatment. Most physicians are aware of the danger of mistaking the acute respiratory alkalosis of salicylate intoxication not uncommonly seen in children for diabetic acidosis.

POTASSIUM AND CALCIUM BALANCE

The relationship between potassium and calcium remains obscure though clinical observations seem to indicate some reciprocal function. Though improvement in the hyperkalemic abnormalities of the electrocardiograph hase been shown to follow the administration of intravenous calcium gluconate, the protective action of calcium against the toxic effects of hyperkalemia seems controversial. Krainin, et al. using dogs could demonstrate no significant evidence to support this hypothesis.[9]

However, the not infreqently observed onset of tetanic symptoms due to low calcium serum levels soon after therapy is started in potassium depletion has been the subject of some conjecture. Bland quotes Rapoport, et al. to describe a postacidotic state following diarrhea therapy in infants. Restoration of water and electrolyte balance may be succeeded by reduction of calcium and phosphorus serum levels severe enough to cause tetany and by concurrent decreases in potassium and phosphate levels. This syndrome is more prone to occur if acidosis has been severe and therapy has been rapid with excessive amounts of sodium bicarbonate. Bland advocates supplementary calcium in spite of normal serum levels in diarrheal acidosis. Calcium should be given separately since it precipitates any phosphate in electrolyte mixtures. Darrow feels that the hypocalcemia in such conditions is often iatrogenic. He claims that he has seldom seen hypocalcemia in such infants when potassium was plentiful. Experiments with normal rats indicate that sodium solutions will produce hypocalcemia only when there is previous potassium depletion. He believes that tetany may be the result of too much sodium bicarbonate administered to patients deficient in potassium.

CONGESTIVE HEART FAILURE

Lown and Levine summarize the electrolyte changes in congestive failure as follows. There is an increase in body sodium though serum levels are moderately lowered due to dilution and intracellular transfer. Chloride concentration is slightly increased, tending to acidosis. Though serum potassium is normal, radioactive isotope technique has proved significant reduction of body stores of that ion. Heart muscle increases in weight with reduction in cellular potassium, phosphorus and creatine. Fasting, anoxia, acidosis, edema and prolonged exercise may induce additional potassium loss.[10]

There is general agreement that significant electrolyte disturbances in congestive failure stem from therapy — the major factors being the salt-restricted diet and diuretic agents, almost all of which can induce potassium deficit. Potassium losses do not occur to as great an extent or as frequently as do those of sodium and chloride, and Bland is of the opinion that sodium and chloride diuretic losses will lead to symptoms before clinically evidenced potassium losses have occurred in most cases. But potassium deficiency should be anticipated in the patient with advanced failure, those who are having repeated predominantly chloride diureses or in patients receiving prolonged ammonium chloride therapy.

The patient suffering heavy losses of chloride may be in a state of hypochloremic alkalosis and on the verge of hypokalemia. Experimental studies with animals show that withholding chloride results in the same electrolyte imbalance as potassium deprivation.[3] Giving ammonium chloride to such a patient causes further potassium loss because the kidney will excrete excess chloride with sodium and potassium the first 2 days of therapy until ammonium ion production begins to increase. Caution is also advised when using ammonium chloride in patients with metabolic alkalosis because of the possibility of misjudging what is actually respiratory acidosis. Even in the absence of respiratory impairment or renal disease, ammonium chloride may produce severe systemic acidosis in the patient with advanced failure, as well as hepatically induced ammonium intoxication in patients with liver damage.

Seldin advocates the use of exchange resins for the therapy of the patient in failure but admits that repeated use of even the newer modifications intended to conserve potassium do result in some depletion of that ion as well as allow the risk of hyperchloremic acidosis due to the ion exchange.[11]

Lown and Levine feel that the major factor in the rising incidence of digitalis toxicity is the increasing dependence of treatment on electrolyte manipulation. Their work indicating that potassium deficiency is related to the threshold of clinical symptoms due to digitalis excess has been universally accepted. They state that in patients with chronic potassium depletion either through the renal or GI route with depression of serum levels, small amounts of digitalis can be fatal, though serum potassium is usually normal during digitalis intoxication.

They believe that postmercurial redigitalization is a state of increased myocardial sensitivity to toxic properties of digitalis resulting from the negative potassium balance precipitated by the diuretic therapy. They claim this can be prevented by giving potassium with the diuretic.

The clinical combination of congestive failure and hepatic cirrhosis is particularly likely to result in potassium depletion because such patients show an inexplicable tendency to lose potassium rather than sodium.[4,12] Excessive aldosterone activity can cause a secondary potassium depletion and alkalosis. Potassium and serum bicarbonate levels should be guarded in those patients with cardiac, nephrotic or cirrhotic edema whose urine shows increased amounts of the adrenal cortical steroid that appears to be responsible for sodium retention.[12]

RENAL DISEASE

Schwartz claims in the course of an extensive review of the effect of potassium on the kidney that renal wasting is not seen in azotemic patients with primarily glomerular damage, but in those with tubular involvement predominant. This may include the diuretic phase of any acute renal insufficiency, however.[12] Furthermore, both Berliner and Schwartz note that an occasional case of hyposthenuric renal insufficiency may show symptoms of potassium depletion due to renal losses.

Functional renal changes which improve with potassium replacement have been reported as resulting from the excessive use of cathartics, chronic diarrhea, ulcerative colitis and in other instances of severe gastrointestinal losses.[4,12] Such renal disturbances included reduced urea clearance, polyuria, lowered glomerular filtration rate and renal plasma flow, and isosthenuria. These changes are reversible, but may require as long as four or five months.

THE SURGICAL PATIENT

The normal pattern of electrolyte response to surgery is a loss of potassium for 2-5 days with little or no change in potassium plasma levels according to Moore and Ball.[13] During this time there is decreased urinary excretion of sodium but plasma sodium levels often fall, especially where trauma is extreme. They state emphatically that though the potassium loss is well tolerated by the average patient for three days,

the period of negative balance should not be allowed to continue over that time. The postoperative period of insufficient caloric intake is at least partially responsible for the electrolyte imbalance and also should not be extended more than 3-5 days post-operatively.

Such a program is not adequate for the previously depleted patient or the complicated surgical case. Harned and Cooke's therapeutic principles for preoperative and postoperative care of infants and children begin with the recommendation that preoperative chemical analysis of plasma or serum and urinalysis including specific gravity precede long or complicated surgery and that any alteration or imbalance be corrected where possible. They recommend repeated studies 12 hours after surgery and at 24-48 hour intervals thereafter as the clinical condition of the patient may warrant.[14]

The surgical care of the patient who has been on a rigid cardiac routine or whose surgery is preceded by a period of starvation or severe vomiting or diarrhea demands that his electrolyte status be investigated and alterations recognized if not corrected prior to operation. Postoperative losses of potassium in such persons must be replaced as they occur. The usual urinary loss during the day of operation will be 50-90 mEq. depending on the nature of the operative procedure and for the three days following surgery will average 20-50 mEq. per day.[13] When possible such losses should be replaced orally — otherwise potassium salt solutions may be added to intravenous fluids at a concentration not exceeding 40 mEq. per liter of fluid. Such infusion must be given slowly over a period of several hours and only when the patient's urinary output is known to be adequate, at least 500 cc. per day, and his plasma potassium is known.

Chloride deficiency and the adrenal response to trauma may quickly produce alkalosis in the patient with chronic potassium deficiency subjected to surgery. Such a state usually results in low plasma potassium levels and low chloride with a high plasma CO_2; these may be accompanied by a high blood urea nitrogen or non-protein nitrogen. Sodium may be normal or low. The patient with a long period of post-operative starvation is especially prone to such a hypokalemic change. Plasma pH and potassium levels should be investigated in the surgical case which suddenly shows a profound prostration and weakness, fever and distention.

Withholding of sodium from the postoperative patient except for replacement of extrarenal losses is generally accepted. Where sodium infusion in reasonable amounts does not elevate low plasma levels, it is advisable to check potassium, CO_2 and plasma protein levels and to add potassium chloride, at least 40 mEq. per day, to infusion fluids. Sodium in amounts concomitant with replacement should be given as chloride, bicarbonate or lactate as indicated by the individual case. Whole blood, protein and caloric replacement must be an integral part of the therapeutic program also. Evans remarks that the restriction of gastric and intestinal drainage to the shortest possible time, adequate daily replacement of sodium and potassium chloride above daily losses before and after operation, and the restriction of intravenous fluid therapy to what is actually required after intelligent appraisal of the individual's need make the surgeon's problem much easier.[15]

There are, of course, cases with refractory low sodium levels which are not due to potassium deficit. Among such is the postoperative hyponatremia in children requiring use of hypertonic saline described by Harned and Cooke and credited to antidiuretic hormone imbalance.[14] Similar syndromes may be seen in intracranial disaese in children and in hypoadrenal conditions.

PYLORIC STENOSIS

The alkalosis associated with pyloric stenosis secondary to peptic ulcer or cancer has been commonly attributed to loss of chloride in excess of base. However, recent findings indicate that this is not the basis for the rise in pH in such patients. Black and Jepson compared balance studies from postgastrectomy recovery records of patients with and without clinical pyloric stenosis and found postoperative retention of potassium as well as sodium and chloride only in the cases with pyloric obstruction.[16] Lans, Stein and Meyer also found potassium deficiency common in pyloric stenosis.[17] Oral administration of potassium salts has been found to diminish the alkalosis even before operative correction of the obstruction. The etiology of potassium loss is not clear in these cases.

Clinically the implications of these findings are two-fold. Heavy administration of sodium and chloride or of other solutions without potassium will add to potassium depletion. The use of ammonium chloride in these patients will enhance potassium loss as well as cause base depletion in general.

Patients with fecal fistula are reported to have potassium deficit reflected by low serum levels, but with normal sodium and chloride balance and no alkalosis. In these cases oral therapy proved fruitless until the fistulae were closed surgically.[15]

POTASSIUM ADMINISTRATION

When all fluids are parenteral Darrow advises that the potassium requirement is from 1-3 mEq. per 100 calories metabolized or 20-30 mEq. per square meter of body surface for twenty-four hours. Total fluid requirements for infants allow 25 mEq. per liter at the most and 60 mEq. per liter for adults. As a general practice it is best to limit the amount per liter of fluid for adults to 40 mEq. and greater concentrations should be given only with constant monitoring of the patient during administration. Deficits should be replaced during at least a 3-4 day period and a longer time if severe. One day's requirement should not be given in less than 4 hours.

We are inclined to think of oral administration as a safe route without question. However, Schwartz warns that in the normal individual 2.5 mEq. per Kg. in a single dose causes a sharp rise in serum level and may cause toxic symptoms if given rapidly. In normal subjects peak serum and excretion levels occur 2-3 hours after ingestion and blood and urine are at initial levels in 4-5 hours. In patients with renal insufficiency as little as 1 mEq. per Kg. in a single oral dose can cause a sudden serum rise and prolonged elevated levels for many hours longer than in control subjects. However, in small doses given at intervals throughout the day, normal persons have been given as much as 600 mEq. daily with no ill effects.[12]

HEMOGLOBIN — AN IMPORTANT BUFFER

Hemoglobin as a buffer is often overlooked as an important factor in electrolyte therapy. In anemia, transfusions provide not only volume replacement but also a medium for taking up hydrogen ions and reducing acidosis. This is especially important in the diabetic case. Bland states that hemoglobin is fully as important a buffer as bicarbonate and accounts for 60% of the CO_2 capacity of whole blood.[4] Occasionally one sees a patient, often with renal insufficiency, whose electrolyte imbalance can be treated only after transfusions have corrected severe anemia. Darrow notes that although restoration of extracellular water and electrolytes is the most important means of correcting the circulatory failure of dehydration and deficit of sodium and chloride, transfusions make this therapy definitely more effective in both experimental animals and patients.[3]

TABLE ONE

Conditions contributing to potassium depletion:

1. Metabolic alkalosis
2. Metabiloc acidosis
3. Periods of starvation
4. Dehydration
5. Anoxia
6. Trauma, stress, or adrenal hormone administration
7. Renal insufficiency
8. Repetitive diuresis — mercurial, acetazoleamide, etc.
9. Severe diarrhea or prolonged overuse of cathartics or enemas
10. Insulin deficiency
11. Pyloric stenosis or obstruction
12. Postoperative states:
    a. Loss due to surgery — 3-5 days
    b. Upper GI losses over 2 liters per day
    c. Lower GI fluid losses
    d. Excessive sodium administration
    e. Heavy purulent discharge

TABLE TWO

Respiratory Alkalosis	Respiratory Acidosis
CO_2 tension decreased. Renal excretion of bicarbonate and sodium proceeds with retention of hydrogen and chloride ions. Muscle cell potassium level — decreased.	CO_2 tension increased. Renal retention of bicarbonate and sodium proceeds with excretion of hydrogen and chloride ions. Muscle cell potassium level maintained.
Metabolic Acidosis	**Metabolic Alkalosis**
CO_2 tension decreased. Renal excretion of metabolic acid proceeds at the expense of sodium and potassium loss. If sodium and potassium deficit develops, bicarbonate concentration cannot be restored since the cations are necessary for hydrogen ion excretion and bicarbonate retention.	CO_2 tension increased. Renal retention of hydrogen ions accomplished by sodium and potassium excretion with bicarbonate. Usually excess sodium is present so only potassium deficit is likely. If potassium is deficient there will be no correction despite abundant sodium excretion.

TABLE THREE

Extrarenal Potassium Losses[13]

Gastric juice	
High free acid	0.5-20 mEq. per liter
Low free acid	15-35 mEq. per liter
Bile	3-15 mEq. per liter
Miller-Abbott tube drainage	2-12 mEq. per liter
Ileostomy	5-15 mEq. per liter (more with ileostomy diarrhea)
Diarrheal stools	15-70 mEq. per liter
Formed feces	10 mEq. per liter
Purulent discharge	10-40 mEq. per day

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THE CHANGING PRACTICE OF OBSTETRICS*

WILLIAM BENBOW THOMPSON, M.D.**

The date was May 9, 1922. M. D., age 25, para i gravida ii, had registered as an obstetrical patient the previous September 12th and was some 18 days beyond her estimated date of confinement when she notified me that contractions had begun at 6:00 a.m. Since she lived only a couple of blocks from the hospital, she was seen at home at about 9:30 and brought in for observation. Labor progressed rapidly and normally, and dilatation was complete at 11:30 a.m. No sedation or other medication had been administered. The membranes were ruptured artificially, and the patient was taken to the delivery room that had been improvised from one of the smaller operating rooms. Drop ether was given with contractions until the head began crowning when it was deepened to a semi-surgical degree. Delivery was accomplished spontaneously without episiotomy. A 1st degree laceration was repaired, the placenta was expressed, 1 cc of Pituitrin was given intramuscularly, and the patient was removed to her room, there to remain for two weeks, although she was allowed to sit up in bed on the 8th day, and out of bed on the 11th. The baby boy weighed 8 pounds 5 ounces, was breast fed, and progressed satisfactorily. The Obstetrical Department of the Henry Ford Hospital was officially opened.

I have given this somewhat detailed account of the first delivery within these walls partly because it has some historical significance on this occasion, but more particularly because it provides a basis on which to discuss the changes in obsterical practice that I have observed in the past thirty-five years. At first glance, one might be pardoned for assuming that no great differences exist between the care of our first delivery and present-day patients. Certainly there are those today who extoll the virtues of "Natural Childbirth" and withhold analgesia and anesthesia. However, no one can deny that great and basic alterations have taken place which have entirely revised obstetrical thinking and practice. Without any idea of discussing all of these — to do so would require a text-book-sized volume — let us take up some of the significant developments that we older practitioners have observed. Let me state at this point that the order in which these are mentioned may not be at all in order of importance.

OPERATIVE TECHNIQUE

Among the chief advances must be listed the improvement in operative technique. Cesarean mortalities in the early twenties were grim indeed. Not only was there extreme danger of infection if labor had been established for any appreciable length of time, but subsequent pregnancies carried grave danger of rupture of the uterine scar, and sterilization usually was advised at the time of the second section. The net result, of course, was that Cesarean was avoided except as a last resort in impossible situations. Hence pubiotomy, a mode of enlarging pelvic diameters that I trust I never will see again; the Walcher position, guaranteed to establish permanent and intractable

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**Clinical Professor of Obstetrics, University of California Medical School, Los Angeles, California.

lumbo-sacral distress; version for cephalopelvic disproportion, in the hope that the after-coming head could be jammed forcibly into the pelvis when it would not enter under uterine contractions; and other procedures that were designed wholly to avoid the hazards of abdominal delivery. Especially to DeLee and Beck must be given credit for setting forth the merits of low-flap Cesareans. At least a moderate trial of labor did not increase the risks involved if Cesarean were finally employed, and the resultant scar was better suited to resist strain in subsequent pregnancies. By the close of the twenties, in the larger centers of population the newer operation was being done by the majority of obstetricians. Later, in the thirties, came the supravesical approach devised at the Hague, so that even neglected and infected parturients could be delivered without too great an increase in mortality. Logically, since operative attentions were made more safe than before, more patients were subjected to Cesarean rather than to brutal vaginal delivery.

One might remark in passing that the improvement in technical phases has not been entirely a net gain. As the tendency increased toward solving all obstetrical difficulties by Cesarean, alternative procedures have been practically abandoned. A whole generation of obstetricians have, for the most part, little experience in the modes of correcting malpositions or the performance of versions. Even breech position is listed as ample excuse for surgical delivery, particularly in primigravidae. The reason for this attitude is quite obvious. At Cesarean, the resident can see clearly the steps of the operation, and when he has his turn at being the operator, his errors are easily corrected. In intrauterine manipulations, however, teaching must be entirely didactic. One can only explain the need for gentleness in handling the babe during version, the accuracy with which forceps are adjusted in mid-pelvic arrest, but the resident cannot observe directly what is being told to him, nor can his own efforts come under scrutiny. Since his training has been better in abdominal delivery than in the more complex vaginal procedures, it is natural and proper for him to solve problems in the manner by which he can best serve his patients. However, in view of the excellence of results attained, I cannot align myself with some of my generation who decry loudly the broadening of the indications for section.

There is a further reliance upon better operative healing that I personally abhor. This involves the assmption that if convalescence has been without fever, uterine tissues are firmly reunited and capable of withstanding the forces of labor in later pregnancies. Hence, if the original indication for Cesarean was not a recurring one, the attendant might feel justified in attempting a so-called "normal delivery". Such reasoning seems to me to be entirely fallacious. If any of those who have reported series of vaginal deliveries following Cesarean have discovered a means of diagnosing accurately the strength of the scar, I have failed to note it. Certainly each report has had a small percentage of failures, with uterine rupture, loss of the baby or uterus or both. Even maternal deaths have been occasioned by this over-anxiety to reduce Cesarean incidence. Moreover, the safe-guards that are advocated are prohibitive in cost, meaning cost in money. To maintain an operation room with nurses and anesthetist in constant readiness is simply too expensive. Even if the hospital is an endowed charitable institution, expenses still have to be paid with money, and in such instances I believe the money could be better utilized. Why chance disaster when a repeat section is even

safer than the original operation, and now presents no bar to the reproductive desires of the mother for additional offspring? At one of the hospitals where I work, and where repeat sections are almsot invariably performed, we have not had a death after repeat Cesarean since the doors were opened more than 25 years ago.

One additional argument against vaginal delivery after Cesarean comes forcibly to mind. I live in an area known as a medico-legal hot-bed. Suits are filed with and without justification. Some years ago, our County Medical Association made up a panel of about 100 members with representatives of all specialties and of general practice, and gave this to the Bar Association as men qualified to confer with plaintiffs' attorneys to aid them in weeding out nuisance suits. At the same time, panel members were instructed to go into court as expert witnesses in cases where the plaintiff had been wronged. I have been on this panel from its inception, and I am sure that my experience in helping to avoid legal actions has not been unique. I am terrified, however, when I contemplate what must be my opinion regarding the loss of a mother, her reproductive organs, or her child when she and her family have relied upon the advise of a physician who has advocated "normal" labor in spite of a Cesarean scar and has had a ruptured uterus in labor.

SEPSIS

From the time of Semmelweiss and Oliver Wendell Holmes, infection has been recognized as the dread killer of postpartum patients. Within my day this danger has lessened to a remarkable degree, but it must never be forgotten that women still die if infection is not prevented. First the chemicals and then the antibiotics have served to rescue many who otherwise would have been doomed. The fear of infection, however, should remain uppermost in the mind of every obstetrician. It is not safe to rely upon the efficacy of curative agents, and particularly it is not safe to ignore or by-pass preventive measures in blind reliance upon modern therapy. There is an ancient fable about an old-time doctor who, when treating a desperately sick patient, was asked if the medicine he had ordered would cure the disease. His answer was that it probably would not, "but it will throw her into fits, and I'm hell on fits". No drug is invariable hell on infection. Here above all, prevention is better than cure.

Better technical procedures already have been mentioned as reducing the incidence of Cesarean infections, but one finds that not always is the preferable technique employed. I have been consulted relative to Cesarean after long hours of ruptured membranes or after vaginal manipulations by those who unblushingly admitted that they intended to perform not an extra-peritoneal approach but a low-flap section, "and I'll load her up with penicillin". Such arguments and attitudes are simply intolerable. If one is not willing to keep abreast with the times in techniques as well as in general measures, then he should not accept obstetrical patients.

HEMORRHAGE

Hemorrhage has been, and is, one of the feared complications of the last trimester, labor, and the postpartum state. Regardless of the efficacy of transfusions, the availability of blood, and the accuracy of laboratory matching tests, the sight of undue

quantities of blood terrifies me. Possibly some of my alarm stems from the fact that I remember when transfusion was not something to turn over to a junior interne, but was a real operation. The first one that I saw involved cutting down on the median artery of the donor and the median basilic vein of the recipient, and then hooking up the two with a paraffin-coated tubing until the donor fainted or the recipent had a nice pink color. Needless to say, there were but few of the students that found they needed $25.00 badly enough to serve as donors. Quite probably we order transfusions nowadays too readily, for such treatment is in itself not without hazard. My personal decision in this is determined by two factors. First, if I would order blood were the patient at the County Hospital, then I want it for my private patient. Second, I want enough to replace the estimated loss plus another pint. Furthermore, I want fresh blood. Unless one is alert, when one orders from the blood bank the technician will reach for the bottle that will be out-dated in a few days. Above all, however, I try to keep in mind a remark I first heard from Edith Potter, that the only ideal blood for a patient is her own.

Since hemorrhage remains one of the chief causes of maternal mortality, are there means by which its occurence may be reduced? I think there are, if one anticipates and prepares for it in advance. Certain patients with certain complications are more prone to bleed excessively, and steps should be taken to speed up active therapy should one's fears be realized. Patients with real or suspected placenta previa or premature separation are obvious candidates, of course, and typing and cross-matching should be done immediately on admission. Others for whom this may be important are those with twins, hydramnios, long and tiring labors, or with a history of excessive bleeding at earlier deliveries. An hour or more is saved in the instituting of transfusion if preparations are already completed. This includes the placing of a large-bore needle and keeping it open with a slow drip of salt or glucose solution when the patient goes to the delivery or operating room. Thus the sudden dismay at finding veins collapsed and shrunken is avoided. In the face of extreme hemorrhage and falling blood pressure, every minute until adequate quantities of blood replace that being lost is of utmost importance. The saving of minutes here means the saving of lives.

TOXEMIAS

In no phase of therapy has there been more improvement than in the care of the toxemic patient. To name over the treatments of the past used to reduce blood pressure and the convulsive state that I have utilized or seen employed makes me realize that I am indeed aged if not altogether antique. Blood-letting in 18th century style I saw once as an interne, when the attendant in attempting to correct a pressure of around 250 mm. inserted a large needle into an arm vein. The stream that gushed out hit the opposite wall of the cubicle. Williams of Hopkins had already declaimed loudly against rapid and shocking emptying of the uterus during the height of the attack, pointing out that the reason for lower mortality in postpartum eclampsia was due to the fact that one could not then terminate the pregnancy by accouchment forcé. However, eclampsia was still the "disease of theories", of which one of the most popular was the hydrops gravidarum theory of Zangemeister. This was maintained by E. D. Plass, the first to head the department here. Empirical treatments, with or

without theoretical basis, were suggested in profusion. The Stroganoff regime, wherein morphine was given until the respirations dropped to 8 or 10 per minute; purges, sweats, and even control of convulsions with chloroform; these seem relics of a past age, as indeed they are. One of the first steps toward logical treatment was the use of intravenous magnesium sulphate, in that both sedation and lessening of blood pressures was accomplished. By this means the mortality of eclampsia at the Los Angeles County Hospital was reduced to less than half its previous level. Incidentally, it may be of interest that this treatment was suggested by an extremely intelligent interne, Dr. Emil Bogen, whose name was barely mentioned in the early reports published by the department chiefs of that time.

Researches of the last decade have been fruitful indeed. Dieckmann, Chesley, Page, and Assali, to name but four of the foremost investigators, have added much to our knowledge of this most perplexing condition. Apresoline and Unitensen are active drugs and have effectively aided clinical management. It must not be assumed, however, that one can now view the eclamptic patient with composure. Toxemias are better prevented than treated, and active care should begin long before the first convulsion demands emergency care. Total results are improved to an encouraging degree, but the millenium is not yet at hand.

ANESTHESIA

It is somewhat paradoxical that anesthesia, in which so much improvement has been accomplished, should now rank as the Fourth Horseman, in that now it rates ahead of embolism as a cause of maternal death. Anesthetic deaths have not increased, of course. To the contrary, anesthetic agents are safer now than ever before. It is merely that other factors in maternal mortality have been reduced to a greater degree. Obstetrical anesthesia is far different in management than surgical attentions. Patients are not often seen at convenient times, or with empty stomachs. In the flurry of moving to the delivery room, an interne, resident, or nurse is pressed into service to administer what is potentially a lethal drug. All too few hospitals maintain a service providing competent anesthetists in the Obstetrical Department at all hours, and where this is attempted, all too often another delivery demands the anesthetist's attentions before the first patient is safely on her way to the ward. The result, of course, is chaos. The attendant, faced with the choice of no anesthesia, drop-ether by a nurse, or no anesthesia at all, often resorts to saddle-block spinal given hurriedly by himself, and then, without having time to see if the effects are all that could be desired, starts to scrub for delivery. It is hardly remarkable that there are some fatal drops in pressure or fatal reactions to the drugs employed.

I prefer conduction anesthesia to general. Given properly, and supervised properly, reactions should be few and far between. Caudal anesthesia seems preferable to me because it can be administered earlier, avoiding the last-minute rush that is so disturbing. I have no quarrel with pudendal block except that it does not alleviate fundal pain and is often given in a hit-or-miss fashion. The tragic thing to me is that a means of giving relief should ever be the cause of grief beyond endurance. Improvement of administration is the urgent problem in anesthesia.

AVOIDANCE OF DELAY

If one looks through the "Year Books" from early in my professional life, he will find a considerable number of abstracts giving the results of series of prolonged labors. In these reports it was evident that lengthy labors meant a rising toll of mothers and infants, and the morbidity rates were high indeed. There were valid reasons for tests of endurance in those days. Low segment operations were just beginning to be advocated, and the hazards of the classical procedure after labor had dragged out for many hours were known to be greater than with further procrastination. "Watchful waiting" was one's rule of conduct, even when one did not know for what he was watching and was merely waiting for disaster to descend upon him and his patient. Happily, those days are over. Present thinking is more concerned with the results of delivery than with the mode of its accomplishment. (One must expect the authorities of the Joint Committee on Accreditation who persists in holding as a tenet of faith that abdominal delivery rates must not exceed four percent regardless of the best interests of mothers or babies.) Hence many of our hospitals require consultation with one of the senior obstetricians if labor has not been productive within 18 to 24 hours. This, I believe, is sensible. Why wait until the mother's resistance has become dangerously depleted and her baby battered into a depressed state before determining that prospects are becoming less favorable each hour? Pleas for earlier intervention are not a recent matter. As far back as 1887, Robert P. Harris of Philadelphia collected a series of 9 women who had been delivered at the horns of cattle, deer, and buffalo, and compared the results — 5 mothers and 5 infants survived — with Cesarean statistics from New York City and in the country at large. As Harris sagely remarked, the better percentages of the animal operators were aided by the fact that their deliveries were done upon patients "when in the full possession of their usual strength and health". It took more than half a century for his warning to receive much recognition, but for the past 15 years or so there has been much less tendency to see just how much agony a woman in labor can endure and still survive. The avoidance of useless and dangerous delay, then, I would set forth as a change distinctly for the better in modern day practice.

It is customary in reviews of this type to remark at some length upon the improvement of prenatal care. I would like to do so here, but I am restrained by the fact that I am not sure as to just what constitutes *good* prenatal attentions. How does one define this? One of my younger conferees sees his patient every other day when they have reached 38 weeks. Another phones his girls every day at this stage of gestation. Either plan would bore me to distraction and must be a great nuisance to patients, but both of these men have sizable practices so it must be impressive. I am disturbed also by the lectures of our professors on this topic. Almost invariably, after dissertating at length upon the need for individualizing one's advice, they then set forth a "routine" to prescribe iron whether the hemoglobin level be high or low. It is "routine" to utilize routines to avoid considering the individual need of the patient at hand. Someway I cannot subscribe to the belief that one's excellence of prenatal care should be measured by the number of pills that one can force down the unprotesting gullets before him. If one must use a routine, let it be that he does the very best he can for the patient he is seeing at the moment.

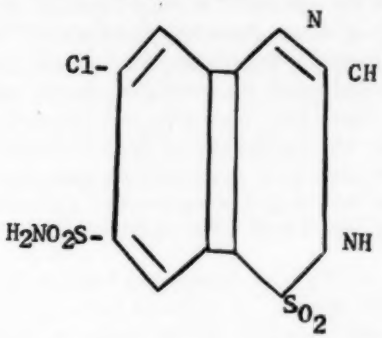
One of the compensations of nearing the end of professional activity is that one can look back and reminisce. As I have tried to show, I have seen vast changes in what has been considered to be proper practice. Nor is the present status fixed or beyond improvement. Tomorrow may see many of our bright ideas outmoded. Some years ago I heard my good friend, Dr. Karl Schaupp of San Francisco talk to a group of medical students about his experiences in the Gold Rush of the Klondike. Karl had climbed Chilkoot Pass, gone through Skagway and down Whitehorse to the headwaters of the Yukon and richer diggings, although he failed to profit materially in their wealth. He made the hardships of weather and terrain stand stark and raw before us. Then he placed before us a comparison of his pioneering days with the lives of his young auditors. Physical frontiers were practically things of the past, he remarked, but in Medicine there were frontiers that were boundless, so that no man of medicine might ever feel himself restricted and hemmed in by lack of new territories to explore. If we be not among those that make the new discoveries, at least let us keep the leaders within our vision and not fall hopelessly behind in the march. Only by making changes is Progress accomplished.

CHLOROTHIAZIDE (DIURIL) — A NEW NON-MERCURIAL ORAL DIURETIC

JOHN W. KEYES, M.D.*

FRANZ J. BERLACHER, M.D.*

Clinical observation concerning a new non-mercurial oral diuretic designated by the generic name — chlorothiazide[1] constitutes this report. It is designated chemically as 6 - chloro, 7- sulfamyl, 1, 2, 4 - benzothiadiazine - 1, 1-dioxide.



It is not primarily a carbonic anhydrase inhibitor[4] but seems to possess the factor of exerting considerable influence on the renal tubular transport of electrolytes, causing a marked decrease in the reabsorption of sodium and chloride, and to a lesser degree potassium and bicarbonate.[2,3,4] The loss of sodium is apparently equal mole for mole to that of chloride.[2] In large doses it is reported[2] that bicarbonate is lost in "appreciable" amounts though never to the point of metabolic acidosis as is the case with carbonic anhydrase inhibitors. Smaller but effective diuretic doses produce only relatively minor loss of bicarbonate.

The drug is rapidly and well absorbed by the oral route with onset of action within two hours and a duration of action of somewhat less than 12 hours.[2,4] The pattern of electrolyte excretion with its marked chloruretic effect and rapid onset suggested a similarity to mercurial diuretics, and further that pretreatment with ammonium chloride might enhance its effect and protect the patient from hypochloremic alkalosis.

Chlorothiazide was employed in several different groups of patients. All were kept on one gram sodium chloride diets and all but two were fully digitalized. All other diuretic agents were stopped except for ammonium chloride. There was a total of 39 patients in the study, all in congestive failure at the time of drug administration.

GROUP I: Patients seen for the first time in congestive failure in whom chlorothiazide was administered in lieu of a mercurial diuretic.

*Adult Cardiology Division, Department of Medicine.

GROUP II: Patients in chronic congestive heart failure who required only sporadic mercurial injections or were taking regular oral mercurial or non-mercurial diuretics.

GROUP III: Patients in chronic congestive heart failure who required regular mercurial injections.

GROUP IV: Patients in refractory state of congestive heart failure in whom regular mercurials did not produce satisfactory diuresis.

Since chlorothiazide was reported[2,3,4] to cause a marked chloruresis, several patients were followed by checking serum electrolyte disturbances. As the study progressed, routine determinations appeared unnecessary since apparent protection against hypochloremia could be provided simply by ammonium chloride administration. The latter was given in one gram doses, four times daily, 3-4 consecutive days a week, as with the mercurial diuretics. The substitution of enteric coated potassium chloride for ammonium chloride, one gram three times daily for three days, may more completely protect the patients from chloride and potassium loss. Chlorothiazide was administered in divided doses ranging from 250 to 1,500 mgms. per 24 hours, 3-7 days a week.

The average dose for long-term therapy was found to be 250 mgms. three times daily at roughly six hour intervals. It appeared that a greater diuretic response was obtained by giving chlorothiazide on a divided dosage schedule, 2-3 times a day than by a single large dose in the morning, and over 3-4 consecutive days each week.

Individual dosage adjustment was, of course, necessary since all patients did not respond in a similar manner, some requiring as much as 1,500 mgms. per day, and others as little as 250 mgms. one day a week. The usual initial dose was 250 mgms. t.i.d., 3-4 consecutive days a week and ammonium chloride 4-6 grams per day the remaining days of the week. Larger doses gave no greater diuresis than smaller effective doses. Subsequent dosage was determined by diuretic response and further need of therapy. There was no evidence of loss of effectiveness of chlorothiazide in the presence of ammonium chloride acidosis, as has been claimed by Maren[5] to be the case with the carbonic anhydrase inhibitor, acetazolamide.

The onset of diuresis was more gradual than with a parenteral mercurial, but continued in a more sustained and less vigorous manner, so that practically all patients agreed it did not cause them the same degree of muscular discomfort and marked frequency of urination with the consequent frequent loss of sleep seen with the shorter and more violent action of a parenteral mercurial.

RESULTS OF STUDY

GROUP I: Patients seen for the first time in congestive failure in whom chlorothiazide was administered in lieu of a mercurial diuretic.

6 cases: 3 arteriosclerotic heart disease — 2 hypertensive cardiovascular disease — and 1 rheumatic heart disease.

Average dosage — 250 mgms. t.i.d. for 3 days.

Average weight loss — 6 pounds at the end of a 3 day period.

GROUP II: Patients in chronic congestive heart failure who required only occasional mercurial injections; or were taking oral mercurial or non-mercurial diuretics.

10 cases: 6 arteriosclerotic heart disease — 3 hypertensive cardiovascular disease — and 10 rheumatic heart disease.

Duration of time followed — 6 weeks to 4 months.

Average time — 2½ months.

Average dosage — 250 mgms. t.i.d. 3 consecutive days each week to 500 mgms. t.i.d. 3 consecutive days each week.

One case received as little as 125-250 mgms. one day a week.

Average weight loss — 5 pounds.

Usual loss — 3 pounds to 8 pounds at the end of each course.

COMMENT: No case failed to be controlled as well as with mercurials (oral and parenteral) or with oral non-mercurial combinations with mercurials.

3 cases much improved over mercurial program.

2 cases receiving regular non-mercurial oral diuretics and occasional mercaptomerin promptly lost 5-8 pounds when placed on chlorothiazide.

1 case on an oral mercurial lost 5 pounds in 4 days after use of chlorothiazide.

GROUP III: Patients in chronic congestive heart failure who required regular mercurial injections.

10 cases: 4 arteriosclerotic heart disease — 1 hypertensive cardiovascular disease — and 5 rheumatic heart disease.

Time followed — 8 to 20 weeks.

Average dosage — 250 mgms. t.i.d. 3 consecutive days each week, to 500 mgms. b.d. or t.i.d.

Prior diuretic — Mercaptomerin or combinations of with chlormerodrin or non-mercurial oral diuretics. Average frequency of parenteral mercury was every 7 to 14 days.

After chlorothiazide — average weight loss — 6 pounds; one case — 12 pounds less.

COMMENT: No case a failure. Every case as well controlled and a great deal more smoothly than on previous diuretic schedule.

GROUP IV: Patients in refractory state of congestive failure in whom regular mercurials did not produce satisfactory results.

3 cases: all arteriosclerotic heart disease.

All were unimproved on mercurial program.

All were unimproved on chlorothiazide.

1 case unimproved then did better when returned to mercurials.

TYPICAL CASE REPORTS

GROUP I: N.G. — 50 year old male was admitted to the Henry Ford Hospital in congestive failure from arteriosclerotic and hypertensive heart disease. He had been on digitoxin 0.2 mgms. q.d. and on a low salt diet. After admission with no change in therapy, his weight remained stable for three days. On the morning of the fourth day, he was begun on 500 mgms. chlorothiazide, b.i.d. At the end of two days he had lost 7 pounds in weight and all signs of congestive heart failure had cleared except for questionable minimal hepatic enlargement. He was continued on chlorothiazide for three more days with the further loss of one pound in weight. Serum sodium, potassium, chloride and carbon dioxide were drawn at this time and all were within normal limits. He was then given 2 cc. of parenteral mercurial to see if any further diuresis could be obtained. There was no change in weight or physical findings.

He was discharged to the Out-Patient Department on maintenance digitoxin and a one gram salt diet, and followed at about monthly intervals requiring parenteral mercurials. After three months he again was given chlorothiazide, 250 mgms. t.i.d., three days a week, and ammonium chloride, 1 gram q.i.d. the other four days of the week. Within 48 hours of institution of chlorothiazide therapy, he lost four pounds in weight and his failure cleared. He has remained out of failure on this program for three months and has required no subsequent mercurials.

GROUP II: B.Z. — 44 year old female with rheumatic heart disease, auricular fibrillation and chronic congestive failure. She had been on digitoxin, ammonium chloride, one gram salt diet, chlormerodrin five days a week and occasionally required a parenteral mercurial. She was begun on chlorothiazide, 250 mgms. t.i.d., three days a week with 4 grams ammonium chloride the other four days of the week. On this program she lost 5 pounds in weight within four days with relief of symptoms and has maintained her improvement for over two months with no need for chlormerodrin or any parenteral mercurial.

GROUP III: H.G. — 70 year old female has been followed at the Henry Ford Hospital for many years with hypertensive cardiovascular disease, auricular fibrillation and chronic congestive failure. She had been on gitalin 0.5 mgms. q.d., one gram salt diet, raudixin, 100 mgms. b.i.d. and for many months had required parenteral mercurials every 7-10 days with intermittent ammonium chloride. Even on this program she had periodic congestive failure with orthopnea and paroxysmal nocturnal dyspnea and edema, usually beginning a few days prior to each mercurial injection. She was begun on chlorothiazide, 250 mgms. t.i.d., 3 days a week and ammonium chloride the other four days of the week.

On this program, she has not needed any mercurial diuretic for five months, and has had no orthopnea, paroxysmal nocturnal dyspnea or edema for the first time in 2-3 years.

GROUP IV: H.F. — 65 year old male with chronic, severe, intractable congestive failure and auricular fibrillation on an arteriosclerotic basis. He has been on digoxin 0.625 mgms. q.d., one gram salt diet, intermittent ammonium chloride and parenteral mercurials, and in the past on chlormerodrin and acetazolamide therapy. He had not been well controlled on any of these programs. Recently he had become almost completely resistant to even parenteral mercurials. He was begun on 500 mgms. chlorothiazide b.i.d., 3 days a week with intermittent ammonium chloride without response. Dosage was increased finally to 500 mgms. t.i.d. every day but still with no satisfactory response. To this program was added parenteral mercurials, 2 cc. every 3-7 days, but with only minimal improvement — that is, a weight loss of from 3-4 pounds with each injection. However, he would regain this weight within 24 to 72 hours. In summary, it was felt that chlorothiazide was of no significant benefit to this patient.

TOXICITY:

Studies to date indicate chlorothiazide has little or no toxicity, however, as with all new drugs, alertness for signs of toxicity must be maintained. Its greatest potential danger seems to be the possibility of producing hypochloremia, hypokalemia or low sodium syndromes, exactly as with mercurials.

Patients with kidney disease should be watched closely and possibly also those with liver function impairment. The drug is apparently primarily excreted by the kidney, being eliminated completely within 24 to 48 hours.[2,4] However, in nephrectomized dogs given chlorothiazide, considerable quantities could be recovered from the biliary tract.[2]

Nausea or vomiting did not occur in any of our patients, the drug being exceedingly well tolerated. No neurologic symptoms such as headache, paresthesias, peripheral neuritis, etc. were found. Skin eruptions were not encountered; hemorrhagic and renal disturbances were checked for, but not present, and are reported absent.[3,4] In short, no evidence of toxicity was uncovered in any of the patients treated in this series.

COMMENTS:

Chlorothiazide appears to represent a significant advance in the diuretic management of congestive heart failure, approaching the effectiveness of parenterally administered mercurials, its decided advantages being:

1). Effective oral administration.

2). Apparent lack of toxicity.

3). Lesser potassium and bicarbonate loss than with carbonic anhydrase inhibitors, and therefore not as likely to produce metabolic acidosis or hypokalemia.

4). Sustained diuretic action over prolonged periods (months) without apparent development of tolerance to the drug and without necessity of increasing dosage, and therefore, more even control of failure than seen with intermittent parenterally administered mercurials.

5). Fairly rapid onset and peak of action.

It should be stated as advisable to respect the potential danger of the drug as with mercurials, in giving it to patients who are salt depleted, and to be cautious in using it without the replacement of chlorides by means of ammonium chloride where the latter can be safely administered.

Study is being given to the possibility of allowing less stringent dietary sodium chloride restriction in patients with congestive failure while being given chlorothiazide, since its rapid saluretic action may prevent the accumulation of fluid.

SUMMARY AND CONCLUSIONS:

Chlorothiazide (6-chloro-7-sulfamyl-1,2,4-benzothiadiozide -1, 1-dioxide) was found to be an extremely effective oral non-mercurial diuretic agent resembling most closely pharmacologically, the mercurial diuretics, particularly in regard to the elimination of sodium and chloride and to a lesser increase, the excretion of potassium and bicarbonate.

Therapeutic dosage varied from 250 to 1,500 mgms. per day with apparent increased effectiveness on a divided dosage schedule. Usual maintenance dose was 500 to 1,000 mgms. three to four consecutive days of the week, alternating with ammonium chloride for its possible potentiating and antihypochloremic effect.

Its effectiveness was not diminished with constant administration, even over a period of several months. Chlorothiazide was found to be an effective replacement for regular oral or parenteral mercurial therapy, and resulted in a smoother control of failure than that seen with intermittent parenteral mercurials in the majority of cases.

Effective for short term diuretic action also (1 to 3 days).

In our limited experience it was of no value in intractable, mercurial resistant cases.

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CHLOROTHIAZIDE AS AN ADJUNCT IN THE TREATMENT OF HYPERTENSIVE CARDIOVASCULAR DISEASE

JOHN R. CALDWELL, M.D.* AND ROY J. KARJALA, M.D.**

It is a remarkable tribute to the group effort in modern medical science that chlorothiazide† in less than one year from the date of reporting its synthesis[1] has been carefully studied and reported upon and made available for wide use in the treatment of edema and hypertension.[2,3]

We report here its use in patients with sustained high blood pressure which has affected the structure or function of the heart as evidenced by cardiomegaly on chest x-ray or left ventricular hypertrophy on EKG. Particular attention was given to the mode of blood pressure measurement[7,8] in order to avoid misleading conclusions which might result if more variable casual blood pressures were used. All of the patients reported here were on moderate salt restriction as a basic part of their antihypertensive therapy, some on 400 mg. sodium diets and some with instructions to avoid highly salted foods and to refrain from the addition of salt at the table or in cooking.

A group of eight patients, who were under treatment with rauwolfia or rauwolfia and hydralazine,†† came in once a week for basal blood pressure determinations for one month prior to starting chlorothiazide as an addition to their program of treatment. Thereafter, basal blood pressure determinations were made once a week after chlorothiazide was instituted. The findings on these patients are summarized in Table 1. The average blood pressure for the group before chlorothiazide was 171 systolic and 105 diastolic. During the period of treatment with chlorothiazide, the average blood pressure for the group was 147 systolic and 89 diastolic, representing a reduction of 24 mm. of mercury systolic and 16 mm. of mercury diastolic for the group. The blood pressure was lowered in every instance. Of the eight patients reported, four continued on the same treatment plus chlorothiazide with blood pressures at lower levels; three were able to discontinue reserpine‡ or reserpine and hydralazine and one decreased the dose of reserpine. All had good blood pressure control on chlorothiazide as an adjunct or alone. The majority of the patients felt well and experienced no undesirable side effects attributable to the drug.

A second group of eight patients in the out-patient clinic were under treatment with ganglionic blocking agents and used home blood pressure recordings as a guide to therapy. These patients and others on ganglionic blocking agents are educated to check their blood pressure in the sitting and standing position just before time for the dose of the ganglionic blocking drug. They are usually instructed to omit the dose of ganglionic blocking drug if the systolic standing blood pressure is below 120 mm. of mercury just before time for the dose. With this home guide to therapy, faithfully followed, few patients encounter harmful effects from an overdosage. The same method of checking blood pressure just prior to the dose of mecamylamine§ was used as a

*Division of Hypertension
**Resident, Department of Medicine
†Diuril supplied by Merck, Sharpe and Dohme
††Apresoline by Ciba
‡Serpasil by Ciba
§Inversine by Merck, Sharpe and Dohme

method of making suitable reductions in the dosage of this drug when chlorothiazide was added to the program. The findings on these patients, all with hypertensive cardiovascular disease treated with mecamylamine and all using home blood pressure recordings as a guide to the dosage of mecamylamine, are listed in Table II. The blood pressure readings listed in Table II represent averages by the week of all standing blood pressures thus measured for a number of weeks before starting chlorothiazide and after starting chlorothiazide. Usually this represented a one month pre-and post-treatment period, although observations extending to two months or more have been essentially the same. The average blood pressures in the pretreatment period were 174 systolic and 109 diastolic with a reduction of 23 mm. systolic and 10 mm. diastolic as the average for the group. The blood pressures were lower in every case when chlorothiazide was used. Three patients were able to discontinue mecamylamine; two were able to reduce the dose by 50% of the previous amount and one to reduce the dose 30% of the previous amount (Fig. 1, 2). Two other patients continued on the same dose of mecamylamine with lower blood pressures and considerable clinical improvement. The average doses of mecamylamine were 28 mg. per day prior to the institution of chlorothiazide and with chlorothiazide the average dose was 13 mg. per day for the group. These patients generally experienced relief from fatigue, less dyspnea, less nocturia and a better work tolerance, indicating a gain from additional reduction of the blood pressure. At the same time, they benefited by a reduction or

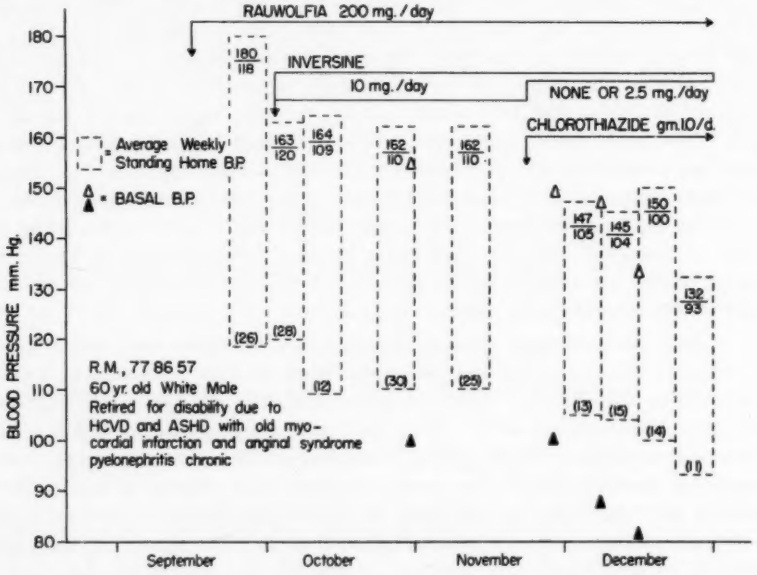

Figure 1

Numbers in parenthesis at the bottom of each dashed rectangle are the number of home blood pressure readings averaged for the week. Home readings were just prior to time for dose of mecamylamine (Inversine), consequently these represent the highest readings. In this case, Basal Blood Pressure determinations in the out-patient clinic were done several hours after the medication was taken at home. Data on this patient is also tabulated in Table II.

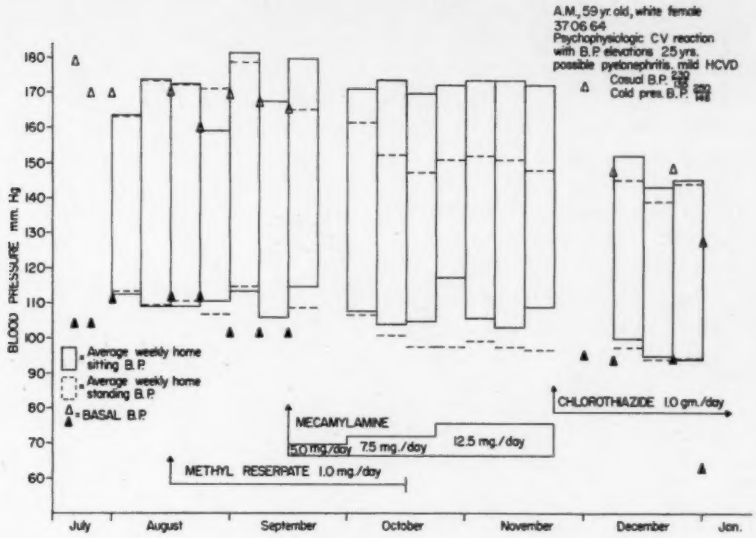

Figure 2

Graphic record of BP from patient with pre-treatment fixed blood pressure of 170/110. Eight months prior to study, treatment with Reserpine had produced depression and fatigue.
Note orthostatic hypotensive effect of mecamylamine with no change in sitting BP. Chlorothiazide alone lowered both sitting and upright BP without much orthostatic difference.

elimination of the undesirable effects of ganglionic blocking agents, particularly a reduction in constipation, dryness of the mouth and blurred vision. These benefits were not restricted to patients treated with mecamylamine but also pentolinium* dosage was reduced or eliminated in other patients not reported in Table II.

In addition to the above sixteen patients, eighteen others were studied in the hospital and the out-patient clinic but sufficient follow-up data is not available for tabulation. Nevertheless, clinical observations on these patients seemed worthy of report.

The drug proved to be particularly useful in a number of patients meriting hypotensive treatment who had moderate or severe hypertensive cardiovascular disease and who could not be treated with customary measures. For example, two patients developed paralytic ileus on relatively small doses of ganglionic blocking drug. One patient, E. C. Case No. 29 47 56, on mecamylamine was able to continue with the drug at a reduced dose of mecamylamine by the use of chlorothiazide without subsequent paralytic ileus. Another patient, Mrs. R. Y., Case No. 63 46 02, had developed paralytic ileus on chlorisondamine† and, at x-ray later, was found to have a stenotic loop of bowel at the terminal ileum. A month after chlorisondamine had been stopped, the patient had a resection of this loop of intestine for regional ileitis. There was a breakdown in the anastomosis and an ileostomy was performed. The patient

*Ansolysen by Wyeth
†Ecolid by Ciba

RAUWOLFIA OR RAUWOLFIA & HYDRALAZINE TREATED PATIENTS

Table I

BEFORE CHLOROTHIAZIDE				AFTER CHLOROTHIAZIDE		
Name, Age, Race, Sex, Case No.	Diagnosis	Treatment	BASAL BLOOD PRESSURE ONCE A WEEK		Change in Treatment	Subjective Results Difference in Blood Pressure
			Weeks	Weeks		
C. R. 56, W Male 11 75 56	HCVD	Rauwolfia* 100 mg./d.	4th 168/106 3rd 166/106 2nd 1st Ave. 167/106	1st 140/88 2nd 122/86 3rd 126/70 4th 150/92 Ave. 134/84	Continue Rauwolfia Chlorothiazide 500 mg. q. 12 h.	Transient paresthesias Felt improved —33/—22
M. S. 36, N Female 76 64 29	HCVD Epistaxis Cardiac insufficiency	Rx irregularly with Rauwolfia SR 100 mg. q.i.d.	4th 3rd 134/84 2nd 142/88 1st 180/120 Ave. 152/101	1st 2nd 118/70 3rd 122/70 4th Ave. 120/70	Continued Rauwolfia Chlorothiazide 500 mg. q. 12 h.	Feeling well No dyspnea —33/—29
J. A. 50, W Male 86 96 36	HCVD	Reserpine 0.5 mg. b.i.d.	4th 158/96 3rd 2nd 1st Ave. 158/96	1st. 118/70 2nd 190/120 3rd 132/90 4th 132/80 Ave. 143/90	Reserpine decreased to 0.5 mg. q./d. Chlorothiazide 500 mg. q. 12 h.	Felt better Enuresis once after 1 month on Chlorothiazide —45/—6
R. W. 51, N Male 72 62 30	HCVD	Reserpine 0.5 mg. q./d. Hydralazine 100 mg. q.i.d.	4th 160/100 3rd 170/104 2nd 170/104 1st 134/90 Ave. 160/100	1st 178/100 2nd 142/90 3rd 132/88 4th 106/74 Ave. 139/85	Continue Hydralazine and Reserpine Chlorothiazide 500 mg. q. 12 h.	Improved Less fatique —21/—15
L. S. 52, W Male 77 96 18	HCVD Encephalomalacia (L) internal capsule	Rauwolfia 100** mg. b.i.d. Hydralazine 100 mg. q.i.d.	4th 180/100 3rd 186/112 2nd 1st 160/90 Ave. 175/100	1st 154/76 2nd 138/88 3rd 178/98 4th Ave. 156/87	Stopped Chlorothiazide after 2 wks. Continued Rauwolfia and Hydralazine. Added Mecamylamine 2.5 mg. q. 12 h.	Nausea and no appetite while taking Chlorothiazide Felt worse —19/—13
F. Y. 64, W Male 71 14 21	HCVD Chronic brain syndrome Arteriosclerosis Azotemia (mild)	Reserpine 0.5 mg. q./d.	4th 3rd 2nd 190/110 1st 174/106 Ave. 182/108	1st 172/114 2nd 147/106 3rd 4th Ave. 159/110	Stopped Reserpine Chlorothiazide 500 mg. q. 12 h.	No complaints Felt well —23/+2
Z. S. 65, W Male 03 78 97	HCVD	Reserpine 0.25 mg. q./d. Hydralazine 25 mg. q.i.d. Caused depression	4th 3rd 170/90 2nd 166/88 1st 154/92 Ave. 160/90	1st 154/84 2nd 148/82 3rd 152/90 4th Ave. 151/85	Stopped Reserpine and Hydralazine Chlorothiazide 500 mg. q. 12 h.	No complaints Felt well —9/—5
J. D. 47, N Male 71 44 21	HCVD	Reserpine 0.5 mg. q./d. Hydralazine 25 mg. q.i.d.	4th 3rd 184/124 2nd 200/130 1st 206/108 Ave. 196/120	1st 154/98 2nd 200/110 3rd 158/98 4th 192/112 Ave. 176/104	Continued Reserpine and Hydralazine Chlorothiazide 1 Gram q. AM	Continued to have headaches Felt well —20/—16
		TOTALS	Ave. 171/105	Ave. 147/89		Ave. —24/—16

*Raudixin Squibb
**Rauwolfia SR Flint Eaton

**Rauwolfia SR Flint Eaton

MECAMYLAMINE TREATED PATIENTS

Table II

BEFORE CHLOROTHIAZIDE					AFTER CHLOROTHIAZIDE	
Name, Age Race, Sex, Case No.	Diagnosis	Treatment	Blood Pressure Ave. by weeks of Standing Home Recorded Blood Pressure Weeks	Weeks	Change in Treatment	Subjective Results and Difference in Blood Pressure
B. W. 48, W Female 86 31 71	Pyelonephritis L. nephrectomy HASCVD Cardiac Insufficiency and Angina	Reserpine .5 mg. b.i.d. Mecamylamine 2.5 - 5.0 mg. q. 8 h.	4th 191/107 3rd 179/110 2nd 182/104 1st 173/102 Ave. 181/106	4th 172/101 5th 170/99 6th 7th Ave. 171/100	Reduced Mecamylamine to 2.5 mg./d. or more often none. Chlorothiazide 500 mg. q. 12 h. Continue Reserpine	Improved. No dyspnea. More strength. —10/—6
S. D. 47, N Male 43 98 64	HCVD Cardiac Insufficiency Creatinine 1.7	Rauwolfia 400 mg. q.i.d. Hydralazine 400 mg. q.i.d. Mecamylamine 20-30 mg. q. 8 h.	4th 196/122 3rd 189/124 2nd 188/113 1st 194/118 Ave. 192/119	1st 135/92 2nd 157/102 3rd 144/90 4th 143/96 Ave. 145/95	Reduced Mecamylamine to 10 mg. q. 8 h. Chlorothiazide 1.0 Gm. q. 12 h. Continue Rauwolfia and Hydralazine	Able to work for first time in 3 mo. Less constipation, less dry mouth and less blurred vision. —47/—24
C. F. 50, W Male 71 50 98	HCVD Duodenal ulcer	Mecamylamine 12.5 mg. q. 8 h.	6th 157/113 5th 150/113 3rd 160/110 1st 160/114 Ave. 157/113	1st 156/110 2nd 146/106 3rd 140/104 4th 137/103 Ave. 145/106	Reduced Mecamylamine to 10 mg. q. 8 h. Chlorothiazide 1.0 Gm. q. AM	Less dyspnea. Less nocturia. Feels better. —12/—7
A. S. 60, N 44 89 44 Male	HCVD	Methyl Reserpate 0.25 mg. q.i.d. Mecamylamine 2.5 mg. q. 12 h.	4th 167/89 3rd 199/103 2nd 216/108 1st 197/108 Ave. 194/102	1st 156/93 2nd 149/90 3rd 4th Ave. 154/91	Chlorothiazide 500 mg. q. 12 h. Continue Methyl Reserpate and Mecamylamine same	Felt much better. —40/—11
R. M. 60, W Male 77 86 57	Pyelonephritis, HCVD, ASHD Old M. I. & Angina	Rauwolfia 100 mg. q.i.d. Mecamylamine 5.0 — 7.5 mg. q. 12 h.	6th 163/120 4th 164/109 2nd 162/110 1st 160/110 Ave. 162/112	1st 147/105 2nd 145/104 3rd 150/100 4th 132/93 Ave. 143/101	Reduced dose of Mecamylamine to none or 2.5 mg./d. Chlorothiazide 500 mg. q. 12 h. Continued Rauwolfia	Improved. Less fatigue. —19/—11
C. McC. 56, W Male 27 90 33	HCVD Asthma	Rauwiloid 6 mg. per day Mecamylamine 20 mg. q. 12 h.	4th 160/84 3rd 163/90 2nd 167/87 1st 166/94 Ave. 164/89	1st 145/89 2nd 143/89 3rd 142/87 4th 141/86 Ave. 143/88	Reduced Mecamylamine to 10 mg. q. 12 h. Chlorothiazide 500 mg. q. 12 h. Continue Rauwiloid	Improved. Less dyspnea. Better work tolerance. —21/—1
S. B. 56, N Female 89 27 39	HCVD Grade III fundi	Mecamylamine 5.0 mg. q. 8 h. (Unable to tolerate Rauwolfia)	2nd 197/136 1st 195/131 Ave. 196/134	1st 184/128 2nd 175/120 3rd 160/115 Ave. 168/118	Chlorothiazide 500 mg. q. 12 h. Increased to 1.0 Gm. q. 12 h. 3rd week Continue Mecamylamine same	Heartburn. Not as tired as before. —28/—16
A. M. 59, W Female 37 06 64	HCVD (mild)	Mecamylamine 2.5 — 7.5 mg. q. 8 h. (Unable to tolerate Rauwolfia)	4th 151/98 3rd 152/99 2nd 151/98 1st 148/97 Ave. 151/98	1st 146/98 2nd 139/94 3rd 145/94 Ave. 143/95	Stop Mecamylamine Chlorothiazide 500 mg. q. 12 h.	Better. No constipation due to Mecamylamine. No drowsiness due to Rauwolfia. —8/—3
		TOTALS	Ave. 174/109	Ave. 151/99		—23/—10

had previously had a severe depressive reaction to reserpine so that all antihypertensive drugs were withheld during several weeks in the hospital at the time of her surgery. She developed signs of progressive left ventricular hypertrophy associated with high blood pressure. Reserpine seemed contraindicated in view of the previous depressive reaction with suicidal trends, ganglionic blocking agents seemed contraindicated in view of the complications associated with her intestinal surgery. Methyl reserpate* parenterally was ineffective in lowering her pressure. We resorted to chlorothiazide and with the use of this drug as the sole antihypertensive agent, her blood pressure came close to normal and remained at satisfactory levels.

Another patient, M. M., Case No. 89 55 57, was a man who had had a depressive reaction to reserpine for treatment of hypertensive cardiovascular disease, and was referred by another doctor for evaluation. Between his first visit and waiting for a hospital bed, he developed a severe gastrointestinal hemorrhage presumably from peptic ulcer. The blood pressure was widely variable in view of repeated hemorrhage and transfusion. When the patient had massive hemorrhage, blood pressure would be very low; transfusions would result in extreme rises in pressure and subsequently further hemorrhage would occur. We did not want to use reserpine in view of his previous depressive reaction and preferred not to use ganglionic blocking agents in view of the intestinal hemorrhage. On treatment with chlorothiazide as the sole antihypertensive agent, along with blood transfusions, his blood pressure gradually stabilized at much lower levels and we were eventually able to control him with small doses of mecamylamine plus chlorothiazide for maintenance.

F. Y., Case No. 71 14 21, was scheduled for a nephrolithotomy for renal calculus. He had had transient cerebral vascular insufficiency, sustained blood pressure elevation and left ventricular hypertrophy by EKG. It seemed desirable to lower his pressure before surgery and in the postoperative period. We were hesitant about giving him rauwolfia since general anesthesia would soon be indicated and we have encountered a number of serious hypotensive reactions during general anesthesia in patients who have been under treatment with rauwolfia derivatives. Treatment with chlorothiazide reduced his blood pressure in the pre-and post-operative periods and appeared to be satisfactory as the sole antihypertensive agent.

One patient, H. B., Case No. 89 33 18, who developed a psychosis about a month following a subarachnoid hemorrhage seemed to be benefited by chlorothiazide. Reserpine was discontinued because he had a severe agitated depression. The use of ganglionic blocking agents was impractical due to his psychotic behavior. Blood pressures were reduced somewhat by chlorothiazide and this was helpful in differentiating his psychotic symptoms from symptoms which could conceivably have been due to hypertensive encephalopathy. One hospitalized patient, Z. R., Case No. 80 95 11, with mild azotemia, showed slight blood pressure reduction when chorothiazide was used for one week as the only treatment. Later, with the addition of intramuscular reserpine, little benefit was gained and considerable drowsiness and stuffiness of the nose resulted. She was treated with chlorothiazide alone and the follow-up period was too brief for a long term evaluation of the benefit but the basal blood pressure was

*Su.3118 Ciba

150/94 after one month on this treatment. Pre-treatment basal blood pressures were all above 170/104.

With respect to side effects and possible toxic effects of chlorothiazide, one patient is described who stopped the drug due to nausea; L. S., Case No. 77 96 18, listed in Table I. Three other patients discontinued the medication due to undesirable side effects. In one patient, this was described as a nausea and heartburn. Another patient after taking the drug for four days frequently had a feeling of pinching in the chest and of blood rushing to the head. She stopped taking the medicine and felt better. Another patient had noticed diuretic effect from chlorothiazide but also dizziness, faintness, and weakness with an exacerbation of neuromuscular chest pains. She discontinued the drug, resumed it one week later and again had the same undesirable symptoms. These last three cases described all had features of neurotic reactions and it is possible that some of the undesirable effects described were in the nature of placebo reaction to the new medication. This seems especially likely in view of the minimum of side effects reported by most other investigators with chlorothiazide.

Three patients with renal insufficiency associated with hypertensive cardiovascular disease were studied in the hospital, maintained on a 400 mg. sodium diet and were given chlorothiazide as part of treatment for their hypertensive disease. One of these patients, T. L., Case No. 89 80 98 had Grade IV fundi and a serum creatinine of 3 mg. percent. The serum phosphorous was 5.4 mg. percent. His highest serum sodium was 137 Meg./L. At a time of maximum chlorothiazide effect at 2 Gm./d. the serum sodium was 127, potassium 2.9, chloride 83 and CO_2 was 27.4. Chlorothiazide in this patient caused a remarkable potentiation in the postural hypotensive effect of inversine and the greatest orthostatic hypotension occurred at a time when the serum sodium, chloride and potassium were lowest. Patient A. C., Case No. 86 73 70 had encephalopathy and renal failure with serum creatinine 7.4 and phosphorous 9.2 mg. percent. She also showed a reduction in serum sodium, potassium, chloride and a rise in CO_2 when chlorothiazide 1 Gm./d. was added to a 400 mg. sodium diet. A. M., Case No. 86 85 15, with recurrent pulmonary edema and renal failure had serum creatinine 7.5, serum phosphorous 9.0 mg. percent. His highest serum sodium was 137. He was on a 400 mg. sodium diet, chlorothiazide and mecamylamine. Diuresis occurred with relief of pulmonary edema. The serum sodium fell to 120, potassium 3.0, chloride 88 and CO_2 increased to 27.6.

There were no apparent adverse effects in these three patients from the depletion of sodium, potassium and chloride which occurred. The average change in serum electrolytes in Meq./L for the three patients with renal insufficiency was for sodium -7.2, for potassium -0.7, for chloride -14.1, for CO_2 +6.4. These electrolyte changes are more marked than those described in most patients on chlorothiazide treatment.[2] This may be due to a combination of renal insufficiency, salt restricted diet and chlorothiazide. Although the depletion of sodium and chloride and potassium was associated with clinical improvement and good hypotensive effects in these patients on concurrent antihypertensive drug treatment, the hazard of salt depletion and low salt syndrome did exist. The changes elicited were reversible readily with cessation of the drug and/or added salt to the diet.

The changes in serum electrolytes of these patients with renal insufficiency were of greater magnitude than those described by Laragh[4] and Ford.[5] Slowly, progressive renal insufficiency as evidenced by a rising creatinine and NPN occurred in two of these three cases but was difficult to relate to undue hypotensive effect or possible nephro-toxic effect of chlorothiazide. In the majority of patients on a higher salt intake with chlorothiazide as reported by Freis[2] no such depletion of sodium, potassium and chloride occurred. A patient reported in Table II, S. D., Case No. 43 98 64, (also charted in Fig. 3) followed a dose of 1 Gm. of chlorothiazide every twelve hours for a period of two months, with no depletion of electrolytes. This patient arbitrarily stopped salt restriction and gained weight not due to edema. It seems likely that this improvement was due to a less monotonous diet and a feeling of well-being associated with a reduction in the dose of his ganglioplegic drug. It is interesting that on a liberal salt intake, this patient continued to have lower average blood pressures than before chlorothiazide. He was able to reduce his dose of mecamylamine by 50 per cent. Three weeks after the start of chlorothiazide at 2 Gm./d. serum sodium was 137, potassium 6.1, chloride 101, CO_2 was 21.7. Six weeks after the start of chlorothiazide his serum sodium was 137, potassium 3.7, chloride 108 and CO_2 was 22.6. It would appear that the danger of salt depletion is not great as long as the oral intake of salt is not severely restricted and as long as there is no unusual loss through repeated vomiting or diarrhea or inability to eat. In the patient

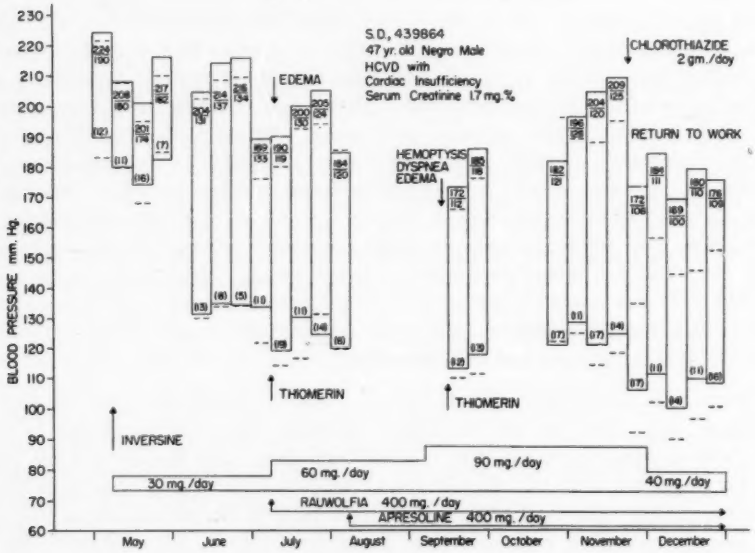

Figure 3

BP record of patient unable to work for 3 months because of recurrent pulmonary edema until chlorothiazide was instituted. Coincident with starting chlorothiazide note: (1) lower average weekly sitting BP (solid rectangles), (2) enhancement of orthostatic hypotensive effect of mecamylamine (dash lines for average weekly standing BP) and, (3) reduction in dose of mecamylamine by more than 50%.

with malignant hypertension T. L., Case No. 89 80 98 salt depletion from chlorothiazide occurred in relation to a reduction in blood pressure with marked orthostatic hypotension. This fact suggests that salt depletion is the principle mode of hypotensive action of this novel diuretic in hypertensive patients. Against this theory is the observation that serum electrolytes are relatively unchanged in most of the patients on chlorothiazide when no salt restriction is advocated. Prolonged use of chlorothiazide at 2 Gm./d. on a relatively liberal salt intake in S. D., Case No. 43 98 64, was not associated with salt depletion. At the same time, there was a maintained blood pressure reduction and also a reduction in the dose of mecamylamine. This suggests some mode of action other than salt depletion for the hypotensive effects.

The major benefit of this new diuretic agent for patients with hypertensive disease is the advantage of being able to reduce or omit less desirable hypotensive agents. By virtue of this change, the weakness, stuffy nose and lethargy some patients experience with rauwolfia may be reduced and the blurred vision, dryness of the mouth, constipation and sexual impotence associated with ganglioplegic drugs may be reduced. Many patients experience a sense of well-being or relief from fatigue. This is little wonder in view of the fact that there is a reduction in rauwolfia and ganglionic blocking drugs as part of the response to the new drug. Studies done elsewhere by Freis[2] and Wilkins[3] have also shown that some of their hypertensive patients treated with chlorothiazide alone had adequate control of hypertension. Also, Freis[2] has studied normotensive patients and found that there is no significant reduction in blood pressure in such cases and that there is a specificity for this drug which is unique. Laragh, et al[4] has shown that in doses up to 8 Gm./d. no side effects were noted and that in thirty patients on usual doses of 1 Gm./d. no toxic effects were noted except erythema in one case. The uric acid was higher in patients studied by Laragh et al[4] and characteristic hypochloremic alkalosis was produced. Ford[5] postulated that the mechanism of diuretic action was due to an inhibition of renal tubular reabsorption of sodium. Schreiner[6] showed in thirteen patients with chronic renal failure that the drug was chloruretic and the poorest clinical responses to the drug were in those patients with chronic renal failure. Perhaps the greatest danger is salt depletion and progressive renal insufficiency.

When chlorothiazide was used in the treatment of 34 patients with hypertensive cardiovascular disease, the drug was found to be an effective antihypertensive agent, usually permitting a reduction and occasionally elimination of the dose of other antihypertensive drugs. In the majority of patients for the period of time studied, it seemed to be of most value as an adjunct to therapy rather than sufficient as the sole form of treatment. According to a clinical estimate results could be graded as follows: excellent 15, good 11, fair 4, poor 4. In a group of eight patients treated with rauwolfia or rauwolfia and hydralazine, the change in the average blood pressure after treatment with chlorothiazide was -24 mm. of mercury systolic and -16 mm. of mercury diastolic for the group. In a group of eight patients treated with mecamylamine the average change in blood pressure was -23 mm. of mercury systolic and -10 mm. of mercury diastolic. In eighteen other patients brief sketches of the most pertinent clinical features are described along with some comment about their response to the drug. Three patients with renal insufficiency were on salt restricted diets and with

chlorothiazide treatment developed salt depletion which was reversible and not deleterious but is noted as a possible hazard of therapy. Side effects were usually absent. In those patients who did develop undesirable reactions, these side effects are briefly discussed. The usual dose was 500 mg. every twelve hours, in the morning and at night. The range of dosage was 500 mg. once daily to 2 Gm. daily. In most cases, dosage was continuous daily. Speculations regarding the mode of action are presented. The time period was short for these observations embracing not more than three months at the most; however, the work of Freis[2] and Wilkins[3] covers larger numbers of patients over longer periods of time, and they report similar results.

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EFFECT OF VIOLENT EXERCISE ON FIBRINOGEN LEVEL

C. PAUL HODGKINSON, M.D.*

Using the serial plasma fibrinogen profile for diagnosis, six conditioned high school athletes were tested to determine if the competitive mile race was of sufficient catabolic influence to cause intravascular conversion of fibrinogen to fibrin. In experimental animals[1] the intravenous administration of thromboplastin-containing solutions can be used to completely deplete the circulating blood of its fibrinogen concentration by stimulating its conversion to fibrin. If administered rapidly, shock and sudden death occur. When given slowly, the animal may survive shock but its blood will be rendered uncoagulable because of depletion of fibrinogen. It may die of hemorrhage.

In clinical obstetrics[2] the syndrome of massive intravascular conversion of fibrinogen to fibrin occurs in premature separation of the placenta[3,4], long intrauterine retention of a dead fetus[5], and in amniotic fluid embolism[6]. Depending upon the speed and degree with which the pathogenic process develops, symptoms of shock, combined shock and hemorrhage, or hemorrhage alone may dominate the symptomatology. In amniotic fluid embolism the process is massive and acute in onset; the result is severe shock and sudden death. In premature separation of the placenta the process is gradual; and both shock and hemorrhage are frequently observed. Gradual conversion of fibrinogen to fibrin occurs in the "dead fetus syndrome" and hemorrhage of uncoagulable blood results. A delayed form of the fibrinogen-fibrin conversion syndrome has been recognized where the terminal precapillary arterioles and the capillaries become occluded in organs whose parenchyma is supplied by arterioles of the "end-artery" type. Resulting necrosis of the cortex of the kidney, the pituitary gland and the adrenal glands have been reported.

In the syndrome of fibrinogen-fibrin conversion, theory holds thromboplastin to be the alleged etiological agent and hyperthromboplastinemia the physiologic mechanism. Shock is assumed to be caused by occlusion of the terminal arterioles with insoluble fibrin gel; and hemorrhage from critical depletion of fibrinogen.

Foster and Whipple[7,8,9,10] (1922) declared fibrinogen to have the following properties:

1. it is a highly dynamic blood protein which is influenced by many stimuli;
2. its sole source of manufacture is the liver;
3. acute catabolic influences and liver destruction cause reduction of its concentration
4. pregnancy, high protein diet and chronic inflammatory states cause its increased concentration;
5. if liver function is normal, complete reconstitution of plasma fibrinogen can occur in 24 hours;

*Surgeon-in-Charge, Department of Gynecology and Obstetrics.

6. it is essential for normal and abnormal cellular repair; and,
7. utilization of fibrinogen stimulates its production.

Foster and Whipple were of the opinion that the concentration of circulating plasma fibrinogen represented the homeostatic balance between normal cellular attrition and hepatic regeneration.

Fibrinogen is a soluble hydro-sol which changes to an insoluble hydro-gel under the influence of thromboplastin. Fibrin, when first formed, is an amorphous net-like gel of sticky consistency which adheres to all endothelial surfaces. In the small pre-capillary arterioles and the capillaries, fibrin causes, first, slowing of the blood flow and then, vascular occlusion from thrombosis. In effect, the process smothers the cells of the parenchyma, much as carbon dioxide smothers fire.

Thromboplastin as a molecular entity has never been isolated. Its presence is recognized by its property of increasing the coagulation of blood. Extracts of tissue, particularly brain, muscle, lung, placenta and gland, are known to be rich in thromboplastin activity. In vivo, cell trauma incites its production. The theory of physiologic tissue repair assumes thromboplastin is essential to the initiation of the fibrin bridge as the first step in the healing process.

Reid, Clark and Rusk[11] stated that the difference between normal physiologic conditions and pathogenic disease was, in many instances, only a matter of magnitude or intensity of the reaction process. In physiology the forerunner of thromboplastin is cellular catabolism. In experimental thromboplastinemia, extracts of macerated animal tissue have served as standard agents for inducing intravascular thrombosis. In pathogenic clinical conditions, cell injury prevails as the common attribute of etiology.

Pathogenic thromboplastin influence has been identified with such acute catabolic influences as abnormal and excessive muscular contraction (eclamptic convulsions and uterine tetany), tissue contusion (crush injury syndrome), cell injury (surgical trauma), and cell destruction (hemolytic transfusion reactions and fetal autolysis of the "dead fetus syndrome").

This project was undertaken to determine if willful muscular exertion of non-pathogenic etiology was a thromboplastin influence of sufficient intensity to initiate intravascular fibrinogen-fibrin conversion.

METHOD

Venous blood samples were collected from six high school boys on each of the two days preceding a one mile race and at the following time intervals after the race: 1 hour, 3 hours, 5 hours and 16 hours. Blood samples of 4.5 cc were obtained by a single sharp venipuncture in every instance and mixed with .5 cc anticoagulant contained in a chemically clean prothrombin tube. Rough handling and forceful syringing of the blood were carefully avoided. The samples were immediately placed in melting ice until the time when all samples would be assayed simultaneously by the same technician using the Wu-Ling colormetric method[12].

Experience had shown that blood samples must be handled in this punctilious manner if curves of diagnostic value were to be obtained. Fibrinogen profiles showing erratic values were of no diagnostic value except to stand as evidence of inept and careless handling of the blood samples.

RESULTS

Complete fibrinogen profiles were obtained in two subjects, incomplete profiles in two and prerace blood samples only, in the other two. Each fibrinogen profile was a uniform curve without a single erratic fibrinogen value. Moreover, a striking consistency of values existed between individual blood samples for any single time interval and the range of variation was less than 39 mg.

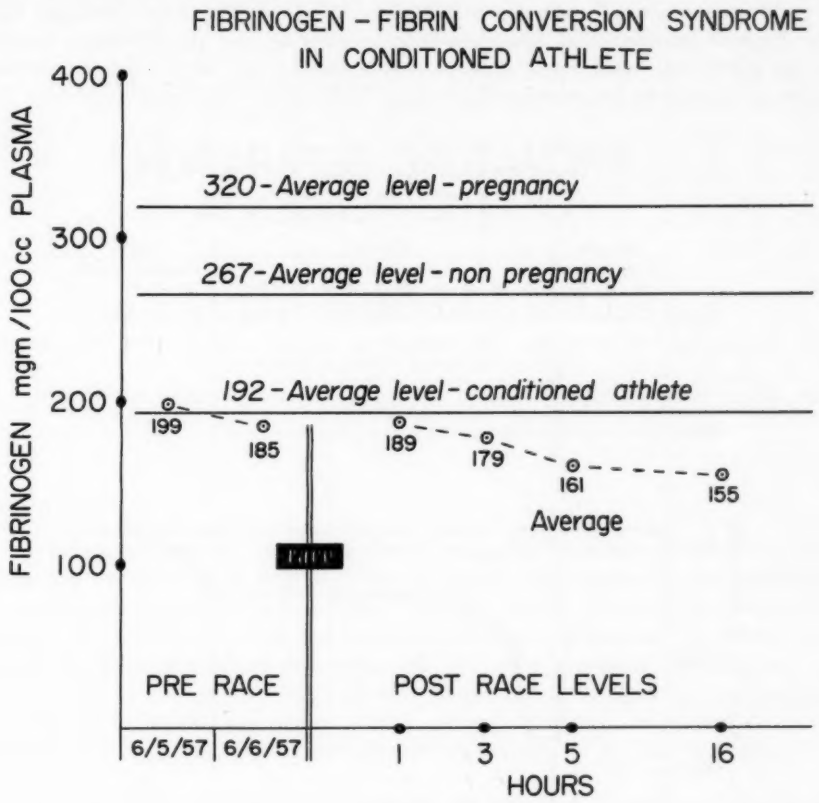

Figure 1

Serial fibrinogen profile constructed from the composite fibrinogen values for 6 conditioned athletes following the mile race.

The pre-race average value for fibrinogen of 198 milligrams is considerably lower than previously determined averages for healthy, non-pregnant adults (267 milligrams) and for healthy obstetrical patients at term (320 milligrams). The cause for this lower average fibrinogen concentration is speculative but may be related to the conditioning process for athletes.

The postrace fibrinogen depression of 18%, maximum at 16 hours, is characteristic of intravascular conversion of fibrinogen to fibrin. This observation suggests that muscular activity of the mile race is a catabolic influence of substantial duration and degree.

Pre-race values averaged 192 mg. per 100 cc of plasma for 12 blood samples. The post-race values showed a steady and uniform fibrinogen depression to a low of 155 mg. per 100 cc plasma at 16 hours (Figure No. 1). The post-race prolongation of the fibrinogen depressing effect was an unexpected phenomenon, and it is regrettable that plans were not made to procure additional blood samples. High school athletes do not make ideal subjects for needle puncturing experimentation, and by the time we were aware that fibrinogen had not reversed its downward trend, the boys were not available.

These results when compared with Figures No. 2 and No. 3 are consistent with the diagnosis of subclinical fibrinogen-fibrin conversion; and the fibrinogen profile is an indictment, based upon circumstantial evidence, that willful muscular effort serves as an exigent thromboplastin influence of moderate magnitude.

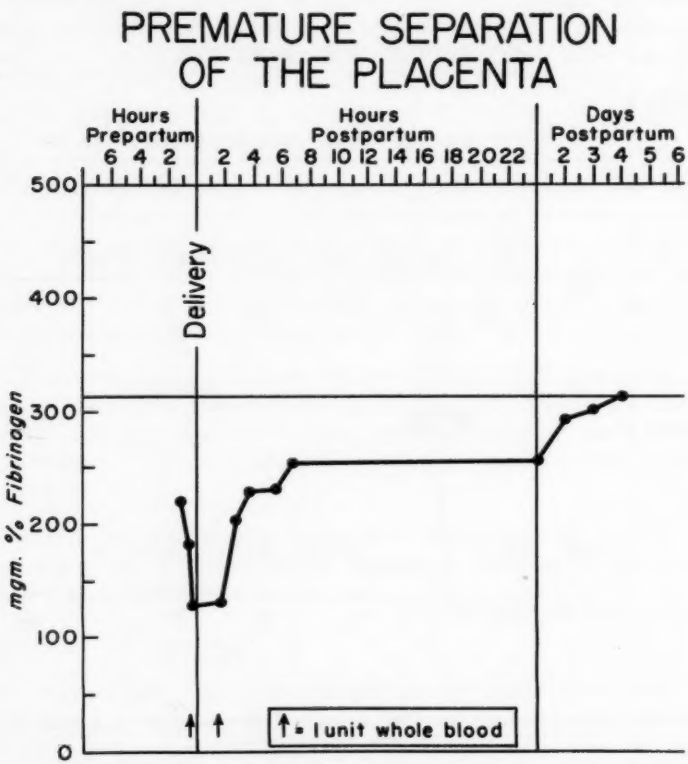

Figure 2

Serial fibrinogen profile of a patient with proven premature separation of the placenta. This patient demonstrated clinical evidence of shock of moderate degree. Her blood formed flimsy clots; however, excessive bleeding did not occur. Her disease was classified as acute, embolic-hemorrhagic, fibrinogen-fibrin conversion.

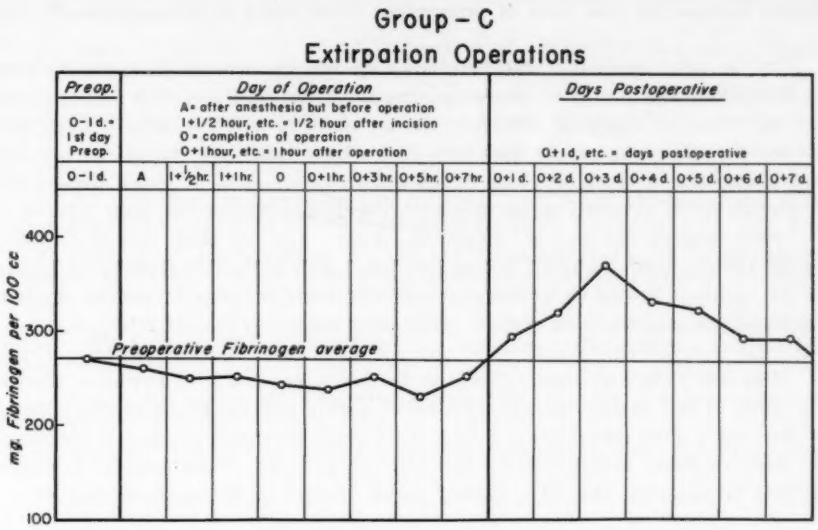

Figure 3

This serial fibrinogen profile is made up of composite fibrinogen values from patients who were subjected to radical gynecologic surgery.

The fibrinogen depression incident to the operative procedure although mild, is protracted. This is followed by a period of hyperfibrinogenemia lasting several days, probably the result of stimulated hepatic activity incident to the post-operative inflammatory phase of the healing process.

COMMENT

The diagnosis of the fibrinogen-fibrin conversion syndrome by means of the fibrinogen profile assumes that fibrinogen depression is preceded by the conversion of soluble fibrinogen to insoluble fibrin; that the degree of fibrinogen depression reflects, in a reciprocal fashion, the degree of *fibrinemia*. Experience has shown that clinical symptoms are seldom discernible until fibrinogen depression approximates the critical level of 90 mg. per 100 cc plasma. Below this level, shock and hemorrhage of uncoagulable blood may occur. Above 120 mg. symptoms are seldom clinically apparent and the condition goes unrecognized unless detected by laboratory means.

The mechanism whereby fibrin thrombi are dissolved in the circulating blood is not known. Considerable emphasis for this responsibility has been placed upon the naturally occurring fibrinolysins. They have been called the "fibrin scavengers of the blood."

It is highly doubtful if the degree of fibrinemia resulting from the muscular effort of the mile race is of sufficient magnitude to adversely affect the patulous vessels of the conditioned young athlete. This is particularly true if additional studies

confirm the relatively low average pre-race fibrinogen values observed in these six athletes, because the total mass of precipitated fibrin would be relatively small.

On the other hand, it is difficult not to speculate possible inimical effects from the fibrinemia resulting from strenuous muscular effort in the subject with partially occluded vessels of marginal efficiency, as may occur in older subjects or those with previous vascular damage. At least two deaths have been reported in runners who were attempting to break the four minute mile record. Also, it would appear that the process might adversely affect athletes whose fatigue barrier had been reduced or eliminated through the use of stimulating drugs. For the desk executive, whose average fibrinogen concentration is probably higher than that of the conditioned athlete, and who subjects himself to occasional intermittent bouts of forceful muscular exertion, and whose age is likely to be over 45 years, the process may be disastrous.

Hess and Fultz[13] reported (1956) on the damaging effects of strenuous exercise in a group of 107 healthy soldiers subjected to a daily physical fitness testing program for two weeks. Four men, all over the age of 30 years, developed physical abnormalities and three of them were unable to complete the program. Four subjects developed transient albuminuria, and 20% showed minor changes in the electrocardiogram.

CONCLUSIONS

A study was undertaken to determine if the muscular effort incident to running the competitive mile race by conditioned athletes was of sufficient catabolic influence to induce depression of the serial fibrinogen profile. The positive results indicated that this form of exertion probably did serve as an exigent thromboplastin influence of moderate intensity.

Although final conclusions are not possible from this preliminary report, the study suggests certain inimical effects which might result from the transient fibrinemia incident to this physiopathologic conversion of fibrinogen to insoluble fibrin.

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MECHANISM AND MANAGEMENT OF THE DUMPING SYNDROME*

WILLIAM S. HAUBRICH, M.D.**

An understanding of the behavior and misbehavior of an abbreviated stomach remnant following partial gastrectomy, the commonest basic procedure employed in the surgical treatment of peptic ulcer disease, is predicated on an appreciation of the physiology of the normal, whole stomach. In oversimplification, the stomach's job may be considered as 3 tasks: a) to act as a receptacle for the ingesta, as a reservoir for the food consumed at a given meal and delivered by the esophagus; b) to initiate digestion of this food within its own lumen and, just as important, to set in motion the digestive processes further in the alimentary canal; and c) to deliver the chyme to the duodenum in the proper amount, at the proper rate, and in suitable consistency. All or part of these important functions are destroyed or altered by the surgeon's knife as he transects the stomach.

Shorn of its greater distal part, the stomach remnant can serve as a very limited receptacle, if as a reservoir at all. It has been deprived of much of its acid bearing mucosa and of the inherent mechanism for stimulating those parietal cells which remain. As the acid activator goes, so goes the pepsin. There remains practically no intralumenal digestion. In the absence of acid (which state of anacidity was the purpose of the operation), the pancreas, liver, gall bladder, and small intestine are hampered in initiating their own important digestive functions. Finally, the material presented by the stomach remnant to the small intestine bears little resemblance to chyme in its consistency, its osmotic concentration, or in its volume or rate of delivery. Viewed in the light of these observations, it is remarkable that the gastrectomized patient exists, indeed often thrives, as well as he usually does.

In terms of the experience of this clinic, the statistics pertaining to which represent a respectable follow-up of almost 5 years, 84.8 per cent of gastrectomized patients were considered† to have emerged from partial gastric resection with good or excellent results as defined by (a) freedom from all gastrointestinal symptoms, (b) subjective well-being, and (c) return to gainful employment or usual activity. A minority (15.2 per cent) have failed to fulfill these criteria. Of these, the greater number consider themselves well by their own estimate and almost all have returned to work. Only 3.8 per cent are classified as "poor" results.

In the last group, 3 patients (1.9 per cent) have exhibited proven or presumed recurrent peptic ulceration. From the "fair" and "poor" categories, 9 have lost an individual average of 22.4 pounds. At the other end of the scale, even in this group, 7 have gained an individual average of 19.9 pounds.

Untoward Post-Prandial Symptoms. In former years, disagreeable post-prandial symptoms were attributed to hypoglycemia. That this erroneous concept was so long entertained is understandable inasmuch as (a) the symptoms often resembled those

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**Division of Gastroenterology.
†By Dr. James I. Baltz, senior associate in gastroenterology, to whom I am indebted for these data.

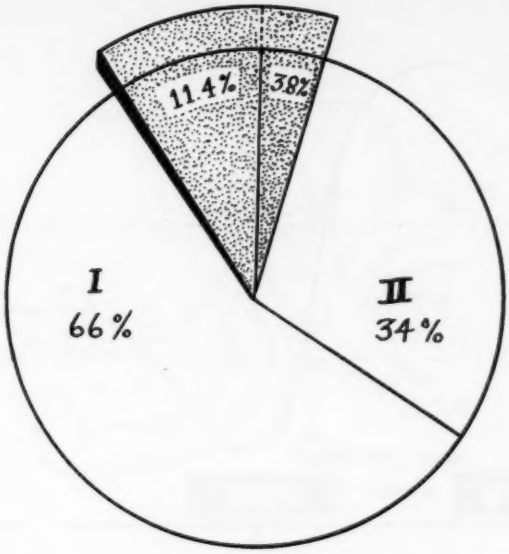

Figure 1

Among 158 gastrectomized patients followed an average of almost 5 years, gastroduodenostomy (Billroth I) was performed in 104 (66 per cent) and gastrojejunostomy (Billroth II) was performed in 54 (34 per cent). Less than "good" results were obtained in 15.2 per cent (strippled segment). A greater than proportional number of "fair" and "poor" results occurred following gastroduodenal anastomosis.

associated with excessive circulating epinephrine such as seen with hypoglycemia, and (b) levels of blood sugar in the post-prandial period were often depressed. With further study (Figure 2) it became apparent that the most intense distress occurred in the earliest post-prandial interval during which the blood sugar curve actually was rising, often to *hyper*glycemic levels. Only later and inconsistently was there "rebound" *hypo*glycemia.

It is now appreciated that there may occur both immediate and delayed post-prandial symptoms. The former are more commonly observed, and only the latter are associated with low blood sugar levels. For an elucidation of the mechanism of the immediate post-prandial symptoms, generally known as the "dumping syndrome", we are indebted to the researches of Machella[1] and, more recently, of Roberts and her associates[2].

Mechanism of the Dumping Syndrome. As we know only too well from the vivid descriptions provided by an unhappy minority of gastrectomized patients, the dumping syndrome may include the following symptoms: a disagreeable sense of upper abdominal fullness and pressure, nausea, vomiting, lightheadedness or near-syncope, palpitation, profuse diaphoresis, abdominal rumbling, and an intense urgency to stool with watery diarrhea. Under varying circumstances and in differing patients, the syndrome may be more or less complete. Elements of the dumping syndrome

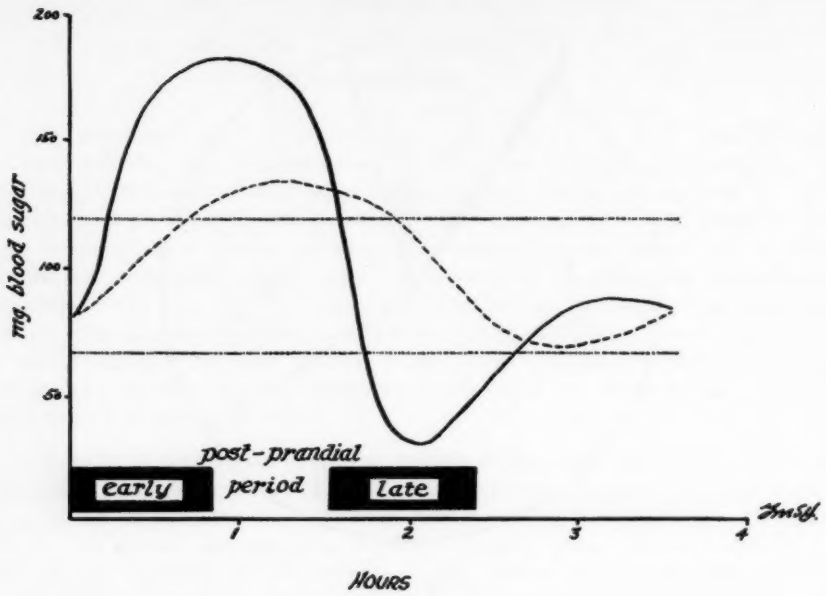

Figure 2

Temporal relationship between immediate and delayed post-prandial symptoms and the concentration of blood sugar in the gastrectomized patient (the usual curve of blood sugar for the individual with an intact stomach is depicted by the broken line).

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occur transitorily in many gastrectomized patients in the early post-operative period. particularly as the diet is first liberalized. Fortunately in only a small minority do symptoms continue unabated; in these few instances the syndrome may persist for months or years.

As the name implies, the dumping syndrome is initiated by the precipitate delivery of ingesta to the proximal small intestine. As such, "dumping" may be observed in the intact stomach from which emptying is unduly rapid and also with intraduodenal or intrajejunal tube feeding in which the rate of flow is excessive. The gastric remnant, following partial gastrectomy, has neither the reservoir capacity nor the pyloric mechanism to restrain the egress of fluid or food.

Machella demonstrated that jejunal distention alone, as by an inflated ballon, often reproduced features of the immediate post-prandial syndrome. It was postulated that such distention served as a stimulus to various autonomic (largely sympathetic) reflexes which, in turn, produced the undesirable symptoms. Such a mechanism does pertain; however, this explanation alone is incomplete.

It is a common observation that certain items of diet are incriminated by the patient more often than others. Among the frequent offenders are concentrated sweets

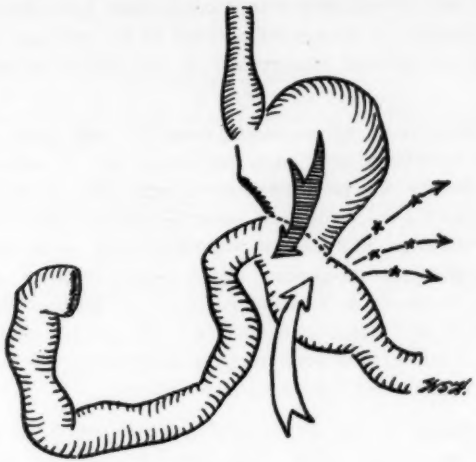

Figure 3

The precipitate and unrestrained "dumping" of food and fluid (shaded arrow) from the gastric remnant into the jejunal lumen results in rapid distention abetted by the inflow of fluid from circulating plasma (white arrow). Symptoms derive from the sympathetic reflexes initated by the distention and the acute fall in plasma volume.

which are actually highly hypertonic in relation to blood. The same effect is produced by the introduction of any substance which may be subject to rapid enzymatic hydrolysis and hence a high osmolarity within the jejunal lumen. Indeed it was demonstrated that the distention of the proximal small bowel resulted, not alone from the ingesta, but also from an outpouring of diluting fluid from the bowel wall itself as a means of inducing isotonicity in the lumenal contents. The only source for this fluid could be, of course, the circulating plasma.

Roberts and her co-workers have amply shown that an acute fall in plasma volume is regularly associated with the dumping syndrome. Further, they have demonstrated that *hypo*volemia ensues within 10 to 15 minutes after the introduction of any hypertonic solution into the jejunem, that it reaches its maximum in 30 to 40 minutes, and that it is spontaneously corrected after 80 to 120 minutes. Hypovolemia invokes sympathetic mechanisms mediated through the pressoreceptor centers in response to a fall in blood pressure and diminution in cardiac output. Although with rapid glycogenesis there is often depression of serum potassium and phosphate, Roberts and her associates found no correlation between the symptoms of the post-prandial period and the levels of these electrolytes.

Associated with the acute hypovolemia are perceptible and reproducible electrocardiographic changes which include tachycardia, flattening of T-waves, alteration in S-T segments, and the occasional appearance of U-waves.

Correction of Post-prandial Symptoms. Quite properly, the avoidance of the dumping syndrome may be considered in 3 categories: a) a discriminating selection of patients for gastrectomy, b) thoughtful selection of the type and technic of operation to the patient, and c) rational management of the patient in whom dumping has been observed.

Because dumping occurs by no means invariably within any group of patients in whom identical operations have been performed and to whom identical stimuli are offered, it is obvious that an individual variation in susceptibility must exist. I share with many observers the notion that dumping is more likely to be manifest in the asthenic, sensitive, nervous, and anxious individual than in the more stable citizen. I am particularly wary of the patient whose history includes past indications of unstable vasomotor reactions as habitual giddiness or fainting. Indeed, I speculate that objective tests might be constructed which could identify these individuals prior to gastrectomy. If I must recommend surgical intervention for these persons, I will try to select an operation other than partial gastrectomy.

With full recognition of the surgeon's skill, I am convinced that the technic of the operation is a contributing factor. Roth and his associates[3] have found that the frequency with which dumping is encountered is proportional to the extent of resection although admittedly the difference is not great. Although no man-made anastomosis can approximate the function of the intact pylorus, I believe that just the right size of stoma and the precise manner in which it is constructed are important in controlling, in part, the rapidity with which the ingested material is presented to the small intestine. It is the majority opinion that the Billroth I (gastroduodenal) anastomosis is less often attended by dumping than the Billroth II (gastrojejunal) anastomosis. Such has not been my own personal observation or the experience of this clinic.

Finally, selection of the operation and the skill of the surgeon notwithstanding, the problem of the patient who has exhibited dumping must be confronted. Sympathetic reassurance and explanation are often helpful, but ultimately the solution must be largely, if not exclusively, by manipulation of the diet. Such diet advice is based squarely on an understanding of the mechanism by which symptoms are produced. From the quantitative view, frequent, small, and relatively dry feedings are required. Qualitatively, the feedings must avoid concentrated sugars or other carbohydrates which may be rapidly hydrolyzed. Protein foods are tolerated well and fats may be helpful in retarding motility and providing maximum calories per unit volume. Anticholinergic medications are traditionally prescribed but are of limited usefulness. The judicious use of mild sedatives is indicated.

SUMMARY

Partial gastrectomy deprives the stomach, in whole or in part, of its normal function in the physiologic scheme of digestion. Precipitate dumping of ingested food and fluid from the stomach remnant produces duodenal or jejunal distention aggravated by an outpouring of fluid from the bowel wall in an attempt to render the lumenal contents isotonic. The resultant acute depression of plasma volume and sympathetic reflexes initiate the complex of subjective sensation which we know as the dumping

syndrome. Control of this untoward sequence requires discriminating selection of the patient, skillful surgery, and rational management of diet.

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ACUTE VIRAL HEPATITIS

Clinical Course and Laboratory Aids in Diagnosis in Thirty-five Consecutive Cases Adrenal Steroid Therapy in Seven Cases

HARVEY L. ATIN, M.D.*

Introduction:

The assertion has been made that the correct diagnosis of acute hepatitis can be made at the bedside by means of a careful history and physical examination in 80 per cent of the patients who present themselves with this disease. With the aid of appropriate laboratory studies this percentage can be increased to 90. The percentage rises to 98 if liver biopsy is also employed.[1] Expectant treatment and observation, along with patience, will add to the accuracy of diagnosis still further. In an attempt to assess the aid from laboratory diagnosis in this disease, and to evaluate the course of the patient and response to treatment, 35 consecutive patients with viral hepatitis and icterus were studied; these patients were admitted to the Henry Ford Hospital during 1956 and 1957. There was no evidence of underlying chronic liver disease in this group. There were 22 males and 13 females and the cases were divided into those due to infectious hepatitis virus (IH) and those due to homologous serum hepatitis virus (HSH). If the patient had received an injection from two weeks to six months prior to the onset of his symptoms, he was considered to have serum hepatitis; we realize that this is not necessarily true, as the IH virus can be transmitted also by the parenteral route. We are also aware of the possibility that the IH and HSH viruses are not necessarily separate strains.

The incubation period calculated from the time of the injection to the onset of symptoms, in the HSH group, varied from 35 to 147 days with an average of 80 days. The patient ranged from 8 to 67 years with an average age of 35 in the IH group compared with 52 in the HSH group. Not only were the patients in the latter group older, but the majority of them suffered from associated disease which necessitated the previous blood or parenteral medication. Whether due to these reasons or due to the inherent greater virulence of the B virus, as some have postulated, the patients with serum hepatitis appear to suffer a more severe form of the disease, as the data will indicate. It has long been said that serum hepatitis has a more insidious onset, and this may be reflected in the longer duration of symptoms prior to admission, i.e., 13.5 compared with 9.9 days.

Method and Results:

Many different methods could have been used to evalute the laboratory data. The method used here was to record the peak abnormality, if any, and determine the number of days this occurred from the onset of symptoms and from the date of admission, and then note how long it took after onset of symptoms for this abnormality to return to normal. The data has been summarized in Table I.

*Resident, Division of Gastroenterology.

Table I

Peak values of laboratory tests in 35 patients with viral hepatitis.

VIRAL HEPATITIS 35 Patients; 22 Males, 13 Females		
Number of Patients	IH 19	HSH 16
Age (yrs.)	8 - 66 (Av. 35)	20 - 67 (Av. 52)
Symptoms Prior to Admission (days)	1 - 28 (Av. 9.9)	4 - 28 (Av. 13.5)
Bilirubin (mg.%)	2 - 15 (Av. 8.1)	6 - 46 (Av. 16.2)
White Blood Count (x 1000)	3.3 - 11 (Av. 5.6)	4.5 - 10.5 (Av. 8.0)
Cholesterol Esters (%)	21 - 58 (Av. 41)	24 - 63 (Av. 39)
Prothrombin Time (%)	Av. 91	Av. 82
Alkaline Phosphatase (Bodansky units)	2.6 - 15.1 (Av. 7.4)	5.0 - 20 (Av. 9.5)
Flocculation Tests		
Ceph. Chol. Flocculation	5/35 less than 3+ throughout course.	
Thymol Flocculation	6/35 less than 3+ throughout course.	
Thymol Turbidity (Units)	3/35 less than 4 throughout course. Av. 13.4	Av. 11.9

Bilirubin:

In HSH the bilirubin peaks were generally much higher (reaching 46 mg. per cent in one case) and, although generally occurring in the first week in the hospital, frequently took much longer (up to 21 days after admission or 31st day of illness). In IH the peaks were lower, rarely over 12 mg. per cent, and usually but not invariably occurred within the first week in the hospital.

White Blood Count:

In no patient was there a white blood count above 11,000. Sevaral patients had white blood counts below 5,000, particularly in the IH group. The absence of prominent leukopenia in the HSH may be due to the longer duration of the illness prior to admission and testing. A white blood count of over 11,000 in uncomplicated viral hepatitis is extremely unusual.

Cholesterol esters:

The esters were usually below 50 per cent in 2/3 of the patients. The sickest patients usually had the lower values, but there is not enough data or correlation to make any further statement in this regard.

Prothrombin Time:

The lowest value was 41 per cent and only two patients had values below 55 per cent. This should not detract from the great prognostic value of a markedly abnormal prothrombin time in hepatitis. A value below 20 per cent is a grave sign of a severely diseased liver.

Alkaline Phosphatase:

There was great variability in the peak values obtained and the number of days required for the peak to be reached. In 24 patients with two or more determinations, five reached values greater than 12 Bodansky units, i.e., approximately 20 per cent reached values in the range of so-called obstructive jaundice.

Flocculation Tests:

Approximately 17 per cent of the patients had one test negative and about 12 per cent had two out of the three tests negative throughout the course of the illness. Only one patient, or about 3 per cent, had all tests negative throughout the course of the illness. Two patients, or about 6 per cent, had all tests negative during the first week in the hospital. Generally the cephalin cholesterol flocculation is the first and the thymol turbidity the last of these tests to be elevated.

Serum Iron and Serum Glutamic Oxaloacetic Transaminase:

According to Rinaldo, in jaundiced patients, values of serum transaminase greater than 250 units and serum iron greater than 200 micrograms are almost diagnostic of hepatitis.[2] Both tests appear to be of particular value early in the disease. About 60 per cent of those tested reached serum transaminase levels greater than 250 units, and 33 per cent serum iron levels greater than 200 micrograms per cent. Since many were admitted some time after the onset of the disease, these percentages are probably far lower than the true yield. The transaminase value in those with serial tests reached the peak about the 16th or 17th day of the illness or within the first week of hospitalization. Since elevated serum transaminase levels did occur in patients with the cholangiolitic variety of hepatitis, a serum transaminase level greater than 250 units, even in the presence of a high alkaline phosphatase and negative flocculation tests, still points strongly to the diagnosis of hepatitis.

Serum Protein:

Total serum proteins and A/G ratios were done on many patients but were all normal. This does not detract from the value of low total protein and particularly a low albumin as an indication of a poor prognosis in patients with hepatitis. Furthermore, although no electrophoretic data was obtained on our patients, it is worth mentioning that a rising gamma globulin is frequently associated with prolonged and chronic hepatitis, while a markedly elevated alpha globulin level is usually indicative of extra hepatic obstructive jaundice or metastatic disease in the liver, rather than hepatitis.[3]

Biliary drainage and Oral Cholecystograms:

There were nine biliary drainages performed. Five of these could be considered abnormal by usual criteria, but only one had calcium bilirubinate pigment. In no case were there both cholesterol crystals and bilirubinate pigment. Three of these nine cases had cholecystograms. Two of these confirmed negative biliary drainage findings. The other case yielded a negative cholecystogram in a patient with micro-calculi in the biliary drainage. Of the five oral cholecystograms performed, (these were performed when jaundice was minimal), only one patient had an abnormal x-ray. That was a patient in whom calculi were demonstrated and who had classical biliary colic while in hospital. It is difficult to draw any conclusions from this data but it is suspected that the biliary drainage must be interpreted with caution in patients with hepatitis.

Complete Suppression of Bile Flow:

Twenty-nine patients had urinary urobilinogen or stool bile examinations on one or more occasions. There were 13 patients or about 40 per cent who exhibited complete suppression of bile flow at some time during their course. Five patients, or about 16 per cent, had complete suppression for greater than 10 days. The longest period of complete suppression in this series was 17 days. The frequency of prolonged biliary suppression in this series again calls to our attention the fact that suppression for more than ten days does not preclude a diagnosis of hepatitis.

Liver Biopsy:

The histological features of viral hepatitis during the acute stage can be simply described as variations and degree of two features: diffuse portal area exudate and parenchymal cell injury.[4] The diffuse portal exudate usually consists of leukocytes and a few eosinophiles. The parenchymal injury may be manifested by focal necrosis and lymphocyte satellitosis, acidophilic bodies, balloon cells, distortion of liver columns in the acute stage and by mitosis, pseudo-acini, lipofuscin pigment in Kupffer cells and bile stasis in the subsiding stage. During the latter part of the illness, the portal exudate and lipofuscin pigment may be the only evidence of liver pathology.

Liver biopsy has been said to be about 90 per cent effective in the differential diagnosis of jaundice.[4] Of the 35 patients studied, ten or about 30 per cent, had a liver biopsy performed. Almost all of the biopsies were done during the resolving stage of the disease, and in only three, or less than 10 per cent, did they appear to be necessary for diagnosis. One biopsy revealed normal liver tissue. The other nine were reported as showing hepatitis. All of these revealed periportal lymphocytes in moderate numbers and a few leukocytes. Four revealed marked focal aggregations of lymphocytes and a few leukocytes in the parenchyma. Only two revealed focal necrosis of liver cells and a similar number showed marked cholestasis. In four patients, there was evidence of liver cell regeneration. There was mild portal fibrosis in two patients, both of whom had HSH with marked cholestasis. There was no evidence of bile duct proliferation, bile lakes and feathery degeneration, so frequently seen in the extrahepatic variety of obstructive jaundice.

Treatment, Course and Prognosis:

All of the patients were treated with high-protein, high-carbohydrate diets, vitamins, and enforced bed rest, during the acute stage of the disease. Some received lipotropic agents, liver extract, and parenteral fluids, carbohydrates and vitamins, as necessary. Two patients received steroids, and their course will be discussed later.

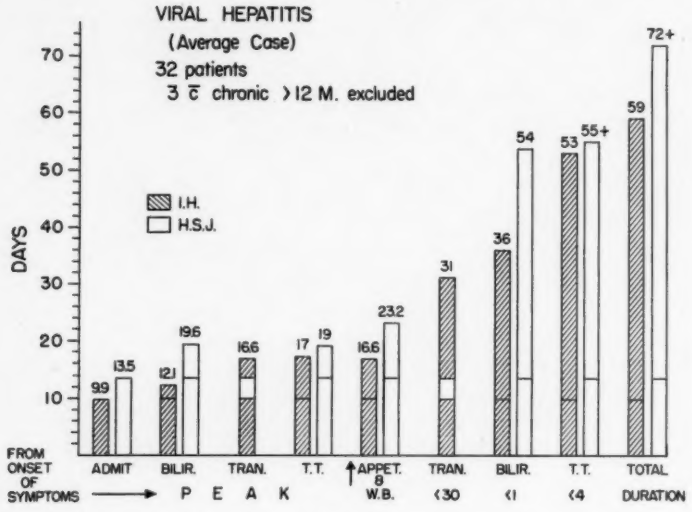

Table II

Laboratory and clinical course in days in 32 patients with acute hepatitis.

Course:

From Table II we note again the increased severity of the disease in HSH compared with IH. The bilirubin, transaminase and thymol turbidity peaks are usually reached in that order during the first week in the hospital. Towards the end of the first week or the beginning of the second week in the hospital, the patient's appetite and sense of well being begin to show a definite improvement. Transaminase appears to be the first of the laboratory tests to return to normal (about 31 days from onset of symptoms) and a persistent elevation in this test usually indicates continued hepatic disease. A secondary rise in the serum transaminase during the recovery phase usually indicates a relapse, and heralds a prolonged or chronic disease. The bilirubin and thymol turbidity take longer periods to reach normal levels. The thymol turbidity is usually the last to return to normal, not doing so until usually the latter part of the second month of the illness in the average case. It should be realized, however, that the thymol turbidity may not return to normal for several months after all the other tests have done so. The total duration of the illness in the average case is about eight weeks in uncomplicated infectious hepatitis, compared with more than ten weeks in HSH.

There were three patients who after one year continued to have elevated flocculation tests and suggestive evidence at least of chronic hepatitis. This percentage of about 10 per cent is higher than usually expected with this disease. If we omit the patients suspected of having chronic hepatitis, we find that two, or six per cent approximately, of the remaining 32 suffered mild transient relapses. This percentage is similar to that reported by Ducci.[5] No patient in this series died.

Although the laboratory is a useful tool in the diagnosis and treatment of viral hepatitis, it still remains but an adjunct to the all important bedside wisdom that can only be obtained by seeing and talking to the patient.

STEROID THERAPY

Of the patients seen at the Henry Ford Hospital with viral hepatitis about 60 per cent are males. During the past two years, seven cases have been treated with steroids. Of these, six or 86 per cent were females. This appears to indicate the disease follows a more severe course in the female patient. Of the seven, three were treated with steroids because of delay in the onset of the recovery phase, i.e. persistent or rising bilirubin and transaminase peaks and persistent complete intrahepatic biliary obstruction. These patients, however, were reported as "feeling and eating well", prior to the onset of steroids, and the steroids were used in an attempt to "break into the disease process". In an attempt to evaluate the course of this group, they were compared with three patients not treated with steroids, who had exhibited complete intrahepatic biliary obstruction for more than ten days. From Table III,

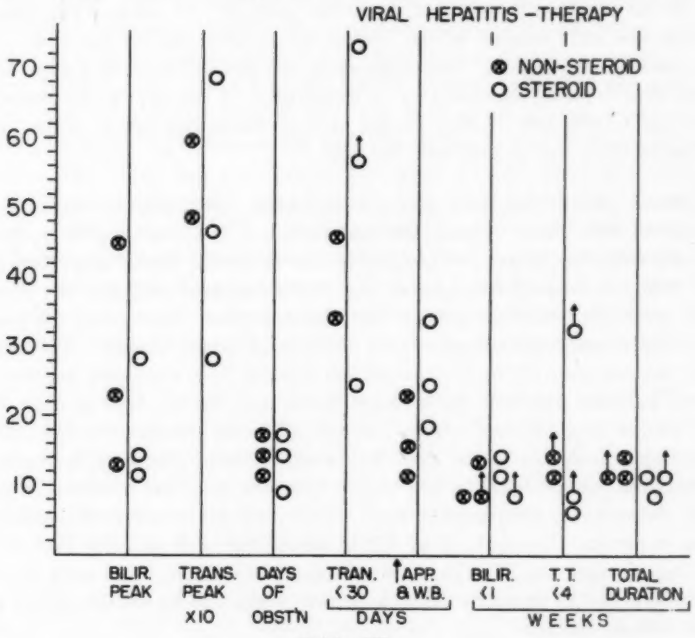

Table III

Comparison of the courses of the steroid and non-steroid treated patients.

we note that the two groups were similar with reference to transaminase peaks and days of complete biliary obstruction. The only significant difference was that two of the steroid group had exhibited transient lethargy five and seven days prior to its use. This indicates to a certain extent a more serious clinical state and will be commented upon later. The apparent lower bilirubin peaks in the steroid group may be due to the effect of steroids in lowering serum bilirubin. There was a definite decrease in liver size and tenderness along with a 50-60 per cent drop in total serum bilirubin within 72 hours of starting the steroids. This was associated with definite evidence of bilirubin in the stool within 48 hours. Although all patients in this group were apparently feeling and eating well, there was a clearcut added surge of well being, to the point of euphoria at times, and increased appetite, within 24 hours. There is some evidence that steroids do decrease the inflammatory reaction in the liver, and may improve bile flow by this method.[6] There is, however, some other unknown mechanism by which the serum bilirubin level is lowered, since this occurs even though bile flow obstruction persists, as noted in the fourth case. The longer period of transaminase elevation in the treated group indicates these drugs do not prevent continued hepatic cell injury and this point should be kept in mind when considering their use in the hepatic coma of viral hepatitis. In spite of the sudden prompt drop in bilirubin with the use of steroids, there is apparently no significant difference in the period required for the total bilirubin to return to normal or in the total duration of the illness in the two groups. Doses of 300-400 mg. of cortisone, or its equivalent dropped gradually over a period of 7-14 days to maintenance levels, appears to be adequate when these drugs are used for the reasons described above. There was one mild relapse in the control group and one relapse with prolonged recovery in the steroid group. Recrudescences can develop in both groups and occur while the dose is being decreased or at termination of therapy in the steroid group. The reported relapse rate is about 20 per cent in the steroid treated group and about 5 per cent in those not treated with steroids.[7]

A fourth patient has been grouped separately. Her disease began in a mild manner about five weeks before. She had carried out strenuous activity during this period, and from her history we considered her to have a severe exacerbation of the disease. While in hospital her appetite and well being decreased and bile disappeared from the stool. Her sensorium, however, remained good. Because of the progressive nature of the disease, she was started on 400 mg. of cortisone daily. The total serum bilirubin dropped from 27 to 21 mg. and her appetite and well being improved within 72 hours. Bilirubin continued to be absent in the stool for the next 14 days, however, and no further drop in total serum bilirubin occurred during this time. Once the bilirubin began to appear in the stool the serum bilirubin gradually decreased. After three weeks the patient is still on 200 mg. of cortisone daily and continues to gradually improve, although her progress has been slightly marred by transient hypomania and persisting euphoria. The early mild fall in serum bilirubin probably had nothing to do with hepatic function. The persistent absence of bilirubin in the stool and absence of progressive fall in serum bilirubin over two weeks can be considered as a partial failure of steroid therapy.

The other three patients who were treated with steroids exhibited progressive

evidence of liver decompensation, manifested by lethargy and restlessness, decreasing well being, appetite, albumin, prothrombin and cholesterol esters, and increasing bilirubin and transaminase. They could be classed as the pre-coma stage of liver decompensation.

Two of these were started on what might be considered average doses of steroids, that is, 400 mg. or less of cortisone or its equivalent. Both exhibited transient improvement in appetite, sensorium and general well being. In one patient, this lasted only 24 hours and the patient subsequently expired on the twelfth day of steroids, despite antibiotics and arginine. This patient had exhibited progressive lethargy for three days, ascites, shrinking liver, and abdominal distention prior to the use of steroids. At post mortem, the patient was found to have diffuse and almost total hepatic necrosis and superficial gastric ulcers, with moderately severe upper gastrointestinal bleeding. The possible role of the steroids in the GI bleeding is of interest, but any answer at present can only be speculative in this regard. The other patient had a fluctuant course with ups and downs of lethargy, state of appetite, and well being for five days prior to steroids. She showed a good response to steroid therapy with marked increase in well being, sensorium, and appetite within 24 hours, and clearing of the obstructive phase with a 60 per cent drop in bilirubin within 72 hours. After 11 days of steroids, when the dose of cortisone was down to 50 mg. daily, she developed a relapse, associated with decreased appetite and well being, rising bilirubin and transaminase, and recurrence of the obstructive phase. The dose was then increased to 800 mg. daily with increase in appetite and well being and cessation of the obstructive phase within 72 hours. The transaminase, which after rising had begun to fall prior to the increase in dosage, continued to fall to normal levels in an uninterrupted fashion. The serum bilirubin level, however, remained elevated without significant change for almost three weeks. The total serum protein continued to fall to a low of 4.8 grams per cent over the next two weeks, in spite of doses of cortisone ranging from 800 to 400 mg. daily. After five weeks on high doses of cortisone, only complicated by mild depression and homesickness, the patient was discharged on 200 mg. of cortisone, eating well, with increasing energy, but still manifesting visible but mild icterus. Although the cortisone resulted in clinical improvement and appeared to stop and reverse the clinical downward trend of the illness, it did not result in sufficient laboratory evidence to indicate improvement in hepatic function and was associated with a clinical exacerbation when the dose was being lowered.

The third patient treated in the pre-coma stage was started on 800 mg. of cortisone daily. She had exhibited progressive hepatic deterioration at another hospital in spite of 15 mg. of meticorten daily. When first seen at Henry Ford Hospital, a "hepatic flap" could already be demonstrated. There was no change in the downhill course with increase in steroid dosage and the patient expired several days later. A request for post mortem examination was denied.

The work of Ducci indicating the value of high doses of cortisone (1 gram of cortisone or its equivalent) in hepatic coma due to viral hepatitis has been an important contribution to the treatment of this almost invariably fatal situation. Evidence to date, however, indicates that lower, more average doses of these steroids

will not alter the outcome of the patient in the coma stage. The doses apprently must be high. Furthermore, the earlier the steroids are used, the greater the chance of survival. The importance of low albumin and prothrombin levels as indicators of hepatic disease does not require re-emphasis at this time. It is interesting to note, however, that only two patients in the original series of 35 exhibited lethargy and both of these received steroids later, after the lethargy had cleared, but when they exhibited a failure to enter the recovery stage of the ailment. Since lethargy occurs when the liver is severely diseased and had appeared from three to seven days prior to the date of beginning of steroid therapy in five out of seven of our patients, we may in this sign find one of the important indicators for those patients who should receive steroids. It is, therefore, suggested that any lethargy, severe enough to be clinically evident, should be met with steroid therapy. The dose at this point should be about 400 mg. of cortisone daily or its equivalent. If the lethargy progresses or clinical state remains stationary after 48 hours, then the daily dose should be increased to 800 to 1000 mg.

Conclusions:

From the work to date, the routine use of steroids in acute, benign, viral hepatitis seems inadvisable.[8] Its use in the patient with persistent, complete, intrahepatic biliary obstruction or cholangiolitic hepatitis is open to question. If the patient appears to be handling the disease well in spite of the absence of bilirubin in the stool, since we are probably not improving liver function or shortening significantly the total duration of the illness with the use of these drugs, the decision to employ them must be weighed very carefully. The possibility of untoward effects of steroid therapy along with a false sense of improvement must be weighed against the possible good results of their use.

Steroid therapy does definitely appear to be indicated in the patient with prolonged and/or progressive liver disease due to acute viral hepatitis. The patient's ability to handle and respond to the disease appears to be the prime consideration here. The important factor is the patient himself, and the decision to use steroids must be made at the bedside where clinical judgment must take precedence over results of laboratory tests. The use of steroids in the pre-coma and coma stages of acute hepatitis has already been discussed and appears well established at present.

The tendency for the disease to run a more severe course in the female and the effectiveness of steroids in many severely ill patients with acute hepatitis brings into focus once more the interrelationship of the endocrines and the liver. Would the use of steroids, other than adrenal, e.g. androgens, be more effectual in the treatment of viral hepatitis?

SUMMARY:

Thirty-five consecutive cases of acute viral hepatitis have been studied in an attempt to determine the average clinical and laboratory course of the disease. The laboratory tests useful in diagnosis and in following the course of the disease are stressed.

Seven cases of acute viral hepatitis treated with steroids are carefully evaluated and the relative value of these agents under various circumstances is discussed.

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4. Gall, E. A.: *Ibid.*, p. 475.

5. Ducci, H., Katz, R., and Allessandri, H.: *Ibid.*, p. 544.

6. Evans, A. S.: *Ibid.*, p. 558.

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8. Ducci, H., Katz, R., and Allessandri, H.: *Ibid.*, p. 549.

The author appreciates the encouragement and assistance of Dr. J. I. Baltz during the preparation of this paper.

INTERNATIONAL SYMPOSIUM

THE MECHANISMS OF HYPERSENSITIVITY

March 27, 28, 29, 1958

The professional staff of the Henry Ford Hospital with the initial advice and support of the Board of Trustees and Dr. Robin C. Buerki, Executive Director, of the Henry Ford Hospital, is sponsoring an international symposium on the subject The Mechanisms of Hypersensitivity. This symposium will be held at the Henry Ford Hospital, March 27, 28, 29, 1958, in the Clinic Auditorium. The program follows and has been prepared with the help of the advisory members:

PROGRAM COMMITTEE

ADVISORY MEMBERS:

Chairman:

Merrill W. Chase	The Rockefeller Institute for Medical Research New York City, New York.
Geoffrey Edsall	Walter Reed Army Institute for Research, Washington, D. C.
Frederick G. Germuth, Jr.	Johns Hopkins University School of Medicine, Baltimore, Maryland.
Herman N. Eisen	Washington University School of Medicine, St. Louis, Missouri.
Bram Rose	McGill University, Montreal, Canada.

LOCAL MEMBERS:

General Chairman:

Joseph H. Shaffer	Division of Allergy, Henry Ford Hospital.
Dwight C. Ensign	Division of Arthritis, Henry Ford Hospital.
Walter J. Nungester	University of Michigan, Professor and Chairman, Department of Bacteriology, Ann Arbor, Michigan.

Secretary:

Gerald A. LoGrippo	Division of Microbiology, Henry Ford Hospital
Clarence S. Livingood	Department of Dermatology, Henry Ford Hospital.
John W. Rebuck	Division of Hematology, Henry Ford Hospital.

PROGRAM

FIRST MORNING SESSION

March 27, 1958 — Thursday, A. M.

8:50 A.M. Remarks of Welcome
Robin C. Buerki, Executive Director, Henry Ford Hospital.

8:55 Announcements
Joseph H. Shaffer
Chairman: *Merrill W. Chase,* New York City

HETEROGENEITY OF ANTIBODIES

9:00 Chairman's Remarks
Merrill W. Chase

9:05 Evidence for Qualitative Differences in Antibodies
David W. Talmage, Chicago

9:30 Quantitative Measurement of Heterogeneity Among Antibodies
Fred Karush, Philadelphia

9:50 Heterogeneity of Diphtheria Antitoxins
Marcel Raynaud, Garches, France

10:10 Discussion Designated
William C. Boyd, Boston, *William Joseph Kuhns,* Pittsburgh, *William H. Taliaferro,* Chicago

10:30 General Discussion

10:45 Intermission

Chairman: *Bram Rose,* Montreal, Canada

DETECTION OF ANTIBODIES IN HUMAN SERA

11:00 Detection of Non-Precipitating Antibodies in Allergic Sera
Alec H. Sehon, Montreal, Canada

11:15 Detection of Antibody Capable of Removing Skin Reactivity in Pollen Extract
Francis C. Lowell, Boston

11:30 Detection of Human Blocking Antibody to Ragweed by Inhibition of a Complement Fixation Test
William B. Sherman, New York City and *Joseph Portnoy,* Chapel Hill, North Carolina

11:40 Approaches to the Problem of Detecting Antibodies
Stephen V. Boyden, Copenhagen, Denmark

12:05 Discussion Designated
Dan Hampton Campbell, Pasadena

12:10 General Discussion

12:30 Luncheon

FIRST AFTERNOON SESSION

March 27, 1958 — Thursday, P. M.

Chairman: *Walter J. Nungester,* Ann Arbor

EFFECTS OF ANTIBODY AND OF ANTIGEN-ANTIBODY COMPLEXES ON INTACT CELLS AND WHOLE ORGANISMS

2:00 P.M. Some Cytologic Effects of Antibodies
Harrison Latta, Los Angeles

2:15 Discussion Designated
John W. Rebuck, Detroit

2:25 *In Vivo* Location of Specific Anti-Organ Sera: Relation to Occurrence of Renal Lesions
Beatrice Carrier Seegal, New York City

2:35 Role of Antibody and of Antigen-Antibody Complexes in Experimental Hypersensitivity
Frederick G. Germuth, Jr., Baltimore

3:00 Role of *In Vivo* Antigen-Antibody Precipitation in Hypersensitivity Reactions
George Edward McKinnon, Salt Lake City

3:10 Intermission

3:30 An Antigen-like Substance obtained from Serum after Disappearance of the Original Antigen
Ludwig A. Sternberger, Army Chemical Center, Maryland

3:45 Immunohistochemical Analysis of Hypersensitivity and Related Lesions
Frank James Dixon, Jr., Pittsburgh

4:05 Discussion Designated
Charles A. Janeway, Boston and *Victor A. Najjar,* Nashville, Tenn.

4:25 General Discussion

FIRST EVENING SESSION

Chairman: *Frank C. MacIntosh,* Montreal, Canada

PERMEABILITY FACTORS

8:00 P.M. Chairman's Remarks
Frank C. MacIntosh

8:10 Serotonin
Sidney Udenfriend, Bethesda, Maryland

8:35 Histamine Metabolism
Richard W. Schayer, Rahway, New Jersey

9:00 The Effect of Corticosteroids upon *in vitro* Blood Histamine Release
Joseph W. Noah, St. Louis

9:10 Mechanisms of Hypersensitivity as Revealed by the Actions of Inhibitors
H. O. Schild, London, England

9:40 General Discussion

SECOND MORNING SESSION

March 28, 1958 — Friday, A. M.

Chairman: *Irwin H. Lepow,* Cleveland

PARTICIPATION OF COMPLEMENT IN ALLERGIC RESPONSES

9:00 A.M. Complement: a Review (Including Esterase Activity)
Irwin H. Lepow

9:20 Dependency of Passive Cutaneous Anaphylaxis on Complement
Abraham G. Osler, Baltimore

9:35 *In Vitro* Models for the Allergic Reaction
Elmer Lewis Becker, Washington, D. C.

9:50 Discussion Designated
Herbert J. Rapp, Baltimore, and *Otto J. Plescia,* New Brunswick, N. J.

10:05 General Discussion

10:20 Intermission

Chairman: *Frank L. Adler,* New York City

AUTO-ANTIBODIES

10:40 Chairman's Remarks
Frank L. Adler

10:45 Auto Immunity in Thyroid Diseases
Ivan M. Roitt and *Deborah Doniach,* London, England

11:05 Autoimmune Hemolytic Anemias
Scott Swisher, Rochester, N. Y.

11:25 Anti-Nuclear 'Antibodies' in Lupus Erythematosus
Halsted Ried Holman, New York City

11:40 Research on L. E. Cell Formation — (Motion Picture)
Roger Robineaux, Paris, France

12:05 Discussion Designated
Stuart C. Finch, New Haven

12:20 General Discussion

12:30 Luncheon

SECOND AFTERNOON SESSION

March 28, 1958 — Friday, P. M.

Chairman: *Herman N. Eisen*, St. Louis

DELAYED TYPE HYPERSENSITIVITY REACTIONS

2:00 P.M. Chairman's Remarks
Herman N. Eisen

2:05 Relationship of the Delayed Hypersensitive State to Antibody Formation
A. M. Pappenheimer, Jr., New York City

2:25 The Cellular Response in forms of Delayed and Immediate-Type Skin Reactions in the Guinea Pig
Melvin H. Kaplan, Cleveland and *Louis Dienes*, Boston

2:40 Discussion Designated
Samuel B. Salvin, Hamilton, Montana

2:50 Transfer of Bacterial Hypersensitivity in Man
H. Sherwood Lawrence, New York City

3:05 General Discussion

3:15 Intermission

3:30 Agammaglobulinemic Humans and Delayed-Type Skin Reactions
Robert A. Good, Minneapolis

3:50 Metabolic Activities of Isolated Intact Lymph Node Cells
Herman N. Eisen, St. Louis

4:05 Histamine in Allergic Responses of the Skin
Theodor Inderbitzin, Zurich, Switzerland

4:25 General Discussion

SECOND EVENING SESSION

HOTEL STATLER

6:30 Reception

7:30 BANQUET

Chairman: *Geoffrey Edsall,* Washington, D. C.

Are We Too Trigger-Happy?
Arnold Ashley Miles, London, England

THIRD MORNING SESSION

March 29, 1958 — Saturday, A. M.

Chairman: *Frederick G. Germuth, Jr.,* Baltimore

IMMUNOLOGIC UNRESPONSIVENESS

9:00 A.M. Immunologic Unresponsiveness to Allergenic Chemicals
Merrill W. Chase, New York City

9:15 Further Observations on Immunologic Unresponsiveness Induced by Type 1 Pneumococcal Polysaccharides
Orton K. Stark, Oxford, Ohio

9:25 Immunologic Unresponsiveness to Protein Antigens
William Oliver Weigle, Pittsburgh

9:35 Competition of Antigens
Frank L. Adler, New York City

9:45 Discussion Designated
Felix Haurowitz, Bloomington, Indiana and *Philip Y. Paterson,* Bethesda, Maryland and New York City

10:00 General Discussion

10:10 Intermission

Chairman: *Chandler A. Stetson,* New York City

TOLERANCE AND REJECTION OF TISSUE

10:25 Transplantation Immunity and Hypersensitivity
Leslie Brent, London, England

10:50 The Role of Antibody in the Rejection of Homografts
Chandler A. Stetson

11:05 Acquired Resistance to Transferred Lymph Node Cells
T. N. Harris and *Susanna Harris,* Philadelphia

11:20 General Discussion

The Mechanisms of Hypersensitivity

Chairman: *Bram Rose,* Montreal, Canada

HORMONES AND ALLERGIC RESPONSES

11:30 Summary of Present Status of Subject
Bram Rose

11:40 Effect of Steroids on Antibody Production *In Vivo*
Marvin Darrach, Vancouver, Canada

12:00 Cortisone and Lymphoid Tissue in Relation to Hypersensitivity
Herbert C. Storek, Rahway, New Jersey

12:20 General Discussion

12:30 Luncheon

THIRD AFTERNOON SESSION

March 29, 1958 — Saturday, P. M.

Chairman: *Herbert C. Stoerk,* Rahway, New Jersey

THE ROLE OF MYCOBACTERIA IN ALLERGIC MANIFESTATIONS

2:00 P.M. Chairman's Remarks
Herbert C. Stoerk

2:05 The Sensitizing and Adjuvant Effects of Mycobacterial Cells and Fractions
Robert George White, London, England

2:20 The Development of Arthritis in the Rat Following Adjuvant Injection
Carl M. Pearson, Los Angeles

2:45 Disseminated Granulomata in the Guinea Pig
Merrill W. Chase, New York City

2:55 Experimental Encephalomyelitis as Prototype of a Class of Allergic Diseases
Byron H. Waksman, Boston

3:10 Discussion Designated
Leon Sokoloff, Bethesda, Maryland, *Charles Ragan,* New York City

3:30 Intermission

International Symposium

Chairman: *Merrill W. Chase,* New York City

SOME FACTORS MODIFYING THE RESPONSE TO ALLERGENS

3:45 The Hereditary Predisposition in Man to Develop Hypersensitivity — A Critical Review
Paul F. de Gara, New York City

4:00 Non-hereditary Factors Affecting Contact Dermatitis in Man
Albert Montgomery Kligman, Philadelphia

4:20 The Metabolism of Different Species in Relation to Their Response to Allergens
David Antony Long, Pittsburgh

4:35 General Discussion

ABSTRACTS OF RECENT PUBLICATIONS OF THE PROFESSIONAL STAFF OF THE HENRY FORD HOSPITAL AND THE EDSEL B. FORD INSTITUTE FOR MEDICAL RESEARCH

***EFFECT OF FERRIC CHLORIDE ON MOBILIZATION OF ACCUMULATED LIVER CHOLESTEROL, IN MICE.** W. T. Beher, W. L. Anthony and G. D. Baker. Proc. Soc. Exp. Biol. & Med. 95:734, 1957.

The effects of ferric ions and cholic acid on the mobilization of accumulated liver cholesterol have been studied in the albino mouse. It was found that dietary ferric ions do not increase rate of liver cholesterol mobilization. Mobilization of elevated liver cholesterol is prevented by addition of dietary cholic acid. Evidence is presented which suggests that cholic acid absorption is not prevented by dietary ferric ions.

NEWER METHODS IN THE DIAGNOSIS OF HYPERPARATHYROIDISM. B. E. Brush and M. M. Block. A.M.A. Arch Surg. 75:953, 1957.

The positive diagnosis of primary hyperparathyroidism is at times very difficult and is dependent on laboratory determinations. The reported levels of serum calcium and phosphorus may be equivocal or inaccurate and vary with dietary intake of calcium and phosphorus and other factors. Other conditions can produce variations in serum calcium and phosphorus which simulate those in hyperparathyroidism. The tubular reabsorption of phosphorus, phosphate deprivation, and intravenous calcium-load tests are of confirmatory value in the diagnosis of this condition. The test for tubular reabsorption of phosphorus appears at this time to be the most definitive of these test.

***CERTAIN METABOLIC EFFECTS OF CORTICOTROPIN, HYDROCORTISONE, PREDNISONE, AND ASPIRIN, IN NORMAL DOGS.** O. H. Gaebler, R. Glovinsky, H. Lees and T. Vitti. Can. J. Biochem. Physiol. 35:1183, 1957.

The losses of weight and nitrogen, produced by daily doses of corticotropin (20 units), hydrocortisone acetate (15 mg.), and prednisone (10 mg.), were studied in normal bitches receiving a constant amount of food equal to that which before therapy had either maintained or permitted it to increase slowly. Loss of weight during therapy was accompained by a decrease in the difference between water intake and urine volume, regardless of whether water intake rose or fell. Weight was restored fairly soon after cessation of therapy with prednisone, but not after corticotropin or hydrocortisone. The probability that calorigenic effects of these substances were involved is discussed. Aspirin (20 grains daily) increased nitrogen output, but weight as well as the difference between water intake and urine volume, increased. The pattern of response to each of the compounds, was, in general, quite similar, but varied in detail in different animals or experiments. Effects of applying hydrocortisone ointment to effected areas in a bitch with dermatitis are also recorded. Prompt improvement was accompanied by losses of weight and nitrogen. Other instances in which the local and general effects of a hormone appear to be contradictory are cited.

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*From Edsel B. Ford Institute for Medical Research.

URINARY STRESS INCONTINENCE IN THE FEMALE: III. ROUND LIGAMENT TECHNIQUE FOR RETROPUBIC SUSPENSION OF THE URETHRA. C. P. HODGKINSON AND W. T. KELLY. Obst. & Gynec. 10:493, 1957.

Success with operations for the relief of urinary stress incontinence varies from 70 to 90%. Urethrocystographic studies have demonstrated the prime importance of reconstitution of the vesical sphincter mechanism if vaginal plastic operations are to relieve this condition successfully. Suprapubic sling operations utilize an equally important general principle: elevation of the urethrovesical junction to a high retropubic position so as to lessen hydrostatic stress on the damaged sphincter. Many prostheses have been tried in a search for the ideal material for use as an elevating sling. Recently use of the anterior vaginal wall as in the technique of Marshall, Marchetti, and Krantz has gained wide acceptance due to the relative simplicity of technique and high degree of success. Certain conditions, however, limit the effective utilization of the anterior wall. As an alternate method of achieving adequate sphincter elevation, a composite suprapubic technique utilizing the round ligaments is described. It has proven successful in 12 cases of severe stress incontinence.

INTERRELATIONSHIP OF PROTHROMBIN AND AUTOPROTHROMBIN I IN THROMBOCYTOPENIA FOLLOWING AMINOPTERIN ADMINISTRATION. S. A. JOHNSON, M. J. CALDWELL AND R. W. MONTO. J. Appl. Physiol. 11:455, 1957.

The poor prothrombin consumption test supported by thrombocytopenic serum has been shown in a previous publication to be due to some activity other than prothrombin — a thrombocytopenic activity. This thrombocytopenic factor or activity can be readily adsorbed on inorganic agents and eluted. The autoprothrombin I values were found to be lower in thrombocytopenic serum than in normal serum. When the patient with thrombocytopenia is treated with aminopterin the prothrombin consumption test remained short while the autoprothrombin I values increased to that of normal serum and then fell again as the treatment was withdrawn. The prothrombin in the serum remained uniformly low throughout the treatment. The relationship of the thrombocytopenic activity and autoprothrombin I as possible derivatives of prothrombin is considered.

*STUDIES ON SUCCINIC DEHYDROGENASE IV. ACTIVATION OF THE BEEF HEART ENZYME. E. B. KEARNEY. J. Biol. Chem. 229:363, 1957.

All preparations of succinic dehydrogenase from beef heart tested, whether soluble or particulate, can be rendered more active by incubation with substances which combine with the active center. The efficiency of malonate, succinate, and phosphate as activators is related to their apparent affinity for the enzyme, as measured by K_1 and K_m values. The activation follows a first order reaction and its rate is profoundly affected by temperature. At 0° the process is not measurable and at 38° is very rapid. The activation energy of the process is 35,600 calories $mole^{-1}$. The increase in activity is accompanied by characteristic changes in the absorption spectrum and small but significant changes in the Michaelis constants for the substrates. The process has been interpreted as a configurational change in the enzyme from a less to more active form.

*From Edsel B. Ford Institute for Medical Research.

EXPERIENCES IN THE USE OF CARDIOPLEGIA (INDUCED CARDIAC ARREST) IN THE REPAIR OF INTERVENTRICULAR SEPTAL DEFECTS. C. R. LAM, T. GAHAGAN, C. K. SERGEANT AND E. GREEN. J. Thoracic Surg. 34:509, 1957.

The closure of ventricular septal defects became possible when methods of by-passing the heart by cross-circulation with human donors and with mechanical pump-oxygenators became available. Originally, these operations were done on the beating heart and the presence of coronary sinus blood and the motion of the heart increased the technical difficulties. Induced cardiac arrest has appeared to be a valuable adjunct in the repair of these septal defects as well as in other intracardiac operations.

Following experimental work with potassium solution and acetylcholine as cardioplegic agents, we adopted the latter. The drug is injected proximal to a clamp on the aorta so that it enters the coronary arteries. The heart is started by removing the clamp to permit the perfusion of oxygenated blood into the coronary arteries.

This technic has been used in 53 operations for the closure of ventricular septal defect. In the favorable group of patients over the age of 3 years, with adequate pump-oxygenator flows, the mortality has been only 5 per cent.

*STUDIES ON SUCCINIC DEHYDROGENASE III. FUMARIC REDUCTASE ACTIVITY OF SUCCINIC DEHYDROGENASE. V. MASSEY AND T. P. SINGER. J. Biol. Chem. 228:263, 1957.

The reversibility of succinic dehydrogenase from yeast and animal tissues has been demonstrated by the constancy of the ratio of activities in the forward and reverse direction from mitochondrial preparations to the highly purified, soluble enzyme and by inhibitor studies. Succinate and malonate are effective competitive inhibitors of the fumaric reductase activity of succinic dehydrogenase. The K_m and K_I values for succinate, fumarate, and malonate in the forward and reverse reactions of the beef heart enzyme have been determined. A differential effect of aging on the succinic dehydrogenase and fumaric reductase activities of the enzyme has been demonstrated and interpreted in terms of different reaction mechanisms operating in the two catalytic activities. Data in the literature on unidirectional fumaric reductases have been reinterpreted in the light of present knowledge of succinic dehydrogenase. A scheme has been presented for the reaction sequences operating during the events of electron transport catalyzed by succinic dehydrogenase.

MANAGEMENT OF IDIOPATHIC THROMBOPENIC PURPURA WITH ACTH, CORTISONE, AND SPLENECTOMY. R. W. MONTO, J. W. REBUCK AND B. E. BRUSH. Postgrad. Med. 22:504, 1957.

It is believed that idiopathic thrombopenia has an immunoallergic basis. There are two forms of the disease, acute and chronic, and the clinical picture and laboratory findings are distinct. Most patients with acute ITP can be successfully treated with ACTH or cortisone. Those with acute ITP in whom there is an incomplete response

*From Edsel B. Ford Institute for Medical Research.

to steroid therapy and those with the chronic form of ITP are subjected to splenectomy. The patients are prepared for operation with ACTH or cortisone.

Successful treatment depends on accurate diagnosis to exclude other forms of thrombopenic purpura. Careful medical observation and vigorous ACTH and cortisone therapy have reduced the need for splenectomy.

A series of 62 adequately treated cases of ITP are cited in which there was only one death, that of a patient who refused splenectomy after cortisone failed to produce an adequate response. In two cases there was only temporary improvement with combined steroid therapy and splenectomy, but symptomatic control was achieved by long-term cortisone therapy. It appears that cortisone gives more satisfactory results than hydrocortisone or prednisone.

INFLAMMATIONS OF THE NONLACTATING BREAST. J. P. PRATT. Am. J. Obst. & Gynec. 74:844, 1957.

In the nonlactating breast the manifestations of inflammation are varied and the responsibility for the management of them is divided among several specialties. Acute infections that involve the subareola present a special problem because they tend to involve the ducts, primarily or secondarily and because they tend to recur unless the effected portion of duct is removed. Tuberculosis of the breast is no longer common but when it occurs it has a characteristic appearance. Fat necrosis which is a result of injury may appear as an inflammation at one phase of its development and later may simulate malignancy. Traumatic mastitis, a mild inflammation of the breast in response to repeated slight trauma, occurs most frequently during the reproductive years and also may be mistaken for infection or malignancy. Although this lesion is tender it lacks other signs and symptoms of infection and differs from malignancy in that it is always tender and usually is discovered because of tenderness. Protection from further trauma reduces the size of the lump while malignancy is not reduced by protection. The patient is usually aware of the source of trauma, which most commonly is point or line pressure of a bra. Chronic cystic mastitis and inflammatory carcinoma of the breast deserve mention because they too may be confused with infections.

FAMILIAL CHOLELITHIASIS, WITH SPECIAL REFERENCE TO ITS RELATION TO FAMILIAL PANCREATITIS. A. J. RINALDO AND J. I. BALTZ. Am. J. Med. 11:880, 1957.

A family with an unusual incidence of cholelithiasis and possible associated pancreatitis was studied. The patients with cholelithiasis had an associated elevation in serum cholesterol and one of these had an inrcease in other components of the serum lipid fraction. The patients with pancreatitis had normal serum lipid levels.

Recent studies dealing with factors which appear to be important in the formation of gallstones are reviewed in relation to the patients presented.

*THE FINE STRUCTURE AND PROPERTIES OF FIBROUS ALUMINA. J. H. L. WATSON, A. V.-F. P. DE SOUZA SANTOS AND J. PARSONS. Kolloid-Zeitschrift 154:4, 1957.

Aluminum hydroxide gels are used as adsorbents for proteins, enzymes and viruses, particularly for preparing vaccines against foot-and-mouth disease.

*From Edsel B. Ford Institute for Medical Research.

The structure and composition of Wislicenus' fibrous alumina was studied by solubility in acids, birefringence, water content, aging in water, x-ray and electron diffraction and by electron microscopy. It was found that fibrous alumina is constituted mainly of amorphous aluminum hydroxide and contains some adsorbed water and some aluminum oxide. The birefringence of the fibers was due to the existence of non-crystalline fibrils of various lengths and about 70-80 A.U. diameter, which were more or less well oriented parallel to the length of the fiber. Amorphous particles, arranged linearly, approx. 50 A.U. in diameter were found to be the ultimate building blocks of these fibrils. These fibrils were often observed to lie together in bundles or fibers about 200-300 A.U. across. Sometimes such groupings of the fibrils did not exist but the whole macroscopic fiber semed to be composed of an oriented bundle of fine fibrils. The binding between particulates in a fibril and between fibrils was very weak. This fact may account for the low density of the material. It has been shown that the formation of fibrous alumina is dependent on the existence of a small amount of water and a small amalgamation on distinct spots on the aluminum surface. The mechanism of the formation of fibrous alumina is suggested as similar to that of anodic films on aluminum. It was observed that strongly amalgamated aluminum or aluminum amalgam itself did not produce fibrous alumina even in air, but formed a grey powder of which the main component was mercuric oxide and aluminum.

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Part II

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HENRY FORD HOSPITAL MEDICAL BULLETIN

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Part II

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1958

A Ten-Year Summary of

The Principal Activities

of

THE EDSEL B. FORD INSTITUTE FOR MEDICAL RESEARCH

1947 — 1957

presented to

The Board of Trustees

and

Officers

EDSEL B. FORD INSTITUTE FOR MEDICAL RESEARCH

INTRODUCTION

This Institute was begun as an activity of Henry Ford Hospital, on May 1, 1947. Two departments, Biochemistry and Physics, were started at that time. Use of the present name was authorized by the Board of Trustees of Henry Ford Hospital, of which Mr. Benson Ford is President, on June 17, 1948. Separate incorporation of the Institute, with Mr. William Clay Ford as President of its Board of Trustees, followed on June 1, 1951. Dr. Robin C. Buerki is the responsible administrative official, having been made a member of the Board of Trustees of the Institute soon after he became Executive Director of the Hospital.

Institute personnel engage in three activities: 1. Research in the basic sciences which have contributed so greatly to advances in Medicine. 2. Research in areas requiring collaboration between men in the basic sciences, and members of the clinical staff. 3. Training of investigators.

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BACKGROUND AND PURPOSE OF THE EDSEL B. FORD INSTITUTE FOR MEDICAL RESEARCH

DR. FRANK J. SLADEN*

Ten years have elapsed since the Trustees of the Henry Ford Hospital established the beginnings of a basic laboratory group and gave to it the name of a man, beloved by everyone who knew him, a man who in his understanding and wisdom and exceptional friendliness, had so much to do with the growth and development of the Henry Ford Hospital itself.

This name seemed extremely appropriate and right, in view of the fact that no one knew the Hospital and the staff better than he did or was more encouraging and inspiring to those who were eager to put the work of the institution on the highest levels of research, education, and medical care.

This name itself gave assurance to the forward-looking staff of those days, that the Institute would grow in its way as differing from the activities of the clinical departments of the Hospital proper, and in due time become indispensable as a basic source of new truths and inspiration to the parent institution. The belief is still as sound as it was ten years ago, that out of pure research in Chemistry, Physics, Bacteriology, Pharmacology, Physiology, and Biology will come the stimulating opportunities for advance in medical knowledge.

The eventful growth in the work of the Henry Ford Hospital has naturally been accompanied by changes in personnel and procedure, conditions which would be expected to result in some loss of appreciation and realization of the background facts upon which certain decisions were made earlier in relation to procedures and activities that still continue.

In some ways the Edsel B. Ford Institute for Medical Research has had this experience and has to some extent struggled against the results of it. This will be more clearly understood as the details of the beginnings are recorded later in this Foreword.

One idealizes pure research today as conditioned by opportunities for devotion to pursuit of truth, in right environment, with adequate equipment and supplies, under capable and inspiring leadership, attracting and training younger research workers by published results as well as by the facilities provided.

The individual reports of the Department of Biochemistry under Dr. Gaebler and of the Department of Physics under Dr. Watson attest how excellently they have built their staffs and employed their opportunities in these ten years of the existence of the Institute.

This has been accomplished in the revamped top floor and portion of the second floor of the Maintenance Building of the hospital, not the most ideal location. Pasteur indeed had much less. The limitations or circumstances of dependence upon the Hospital for space and maintenance enable knowing ones to understand the situation.

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*Consultant in Medicine, Henry Ford Hospital. Physician in Chief, 1915-1952.

This is particularly understandable because, in the same year of the establishment of the Institute, the Henry Ford Hospital began the planning and ultimate financing and erection of the seventeen story Clinic Building. The organization of the Institute, the attracting and interesting of the best personnel, the maintaining of the spirit of fruitful endeavor and of the morale of the staff members have been accomplished in spite of these difficulties.

The Hospital was opened in September, 1915, and a recess occurred from 1918 to 1922 when the staff participated in World War I and the buildings became U. S. Army General Hospital Number 12. From 1922 to 1952, the growth of the Hospital was extraordinary considering the many unusual and unorthodox features of its operation. We refer to the full-time employment of the staff, the substitution of a quality of private patient-private doctor outpatient system for the more orthodox dispensary plan, the previously unheard of deposit in advance of inpatient admissions, the appointment card for outpatient visits, and many other at that time unorthodox procedures. However, many of these have been adopted elsewhere and are now in more or less general use.

The contributions of Mr. Henry Ford and Mr. Edsel Ford to the story of hospital procedures has not yet had the recognition it deserves. This is a subject upon which the writer is already busying himself.

To properly picture what was behind the establishment of the Edsel B. Ford Institute for Medical Research, one must go back to the turn of the century, or even earlier.

When Dr. William Osler left Baltimore and the Johns Hopkins Hospital in 1905 and became the Regius Professor of Medicine at Oxford, he expressed as his greatest pride the fact that he was responsible for the introduction of bedside teaching of medical students. This began with the first class in the Medical School of Johns Hopkins University graduating in 1897.

With this beginning the University Hospitals of the country became the centers of medical education and research. A great contrast existed in the so-called non-university hospitals. The medical school faculties were responsible for this. They supplied the doctors who took the responsibility of the medical work in the University Hospitals. Not a small factor, by any means, was the proximity of the laboratories and the teaching of the basic sciences in the medical schools. Solid foundation was given to the clinical staff work of these hospitals by the research work of those in the basic science fields.

From 1915 to 1935, the staff of the Henry Ford Hospital grew not only in numbers but also in the spirit of teaching. This was related to the realization that many of its staff members had previously been members of such faculties, and had experienced the stimulations and satisfactions of teaching. They wanted to teach. This led in time to the development of a curriculum in the Henry Ford Hospital for men in training, for the intern in his first year, for the second and third year men, Assistant Residents and Residents, and for the fourth and fifth year men in advanced work in one field or another.

Thus a faculty arose composed of the "permanent" staff men in charge of divisions and departments and their associates as contrasted to those mentioned above who were in training. This amounted to a faculty of seventy-five men carrying out a curriculum for one hundred and twenty-five of the "temporary" staff men. It was no small task considering the fact that this was a non-university hospital designed to meet community needs. Every staff member was under demand to care for inpatients and outpatients, who expected personal consideration and service.

The so-called academic freedom was an unknown item in such a situation.

For a time, an arrangement existed whereby men could earn a degree of Master of Science in Surgery, for instance, from the University of Michigan, by attending certain seminars and teaching activities in Ann Arbor and giving evidence of work accomplished in the Henry Ford Hospital, in addition to writing an acceptable thesis at the end of the year.

The Hospital thus played the part of the field laboratory where the work was done in a creditable way by the candidate under the supervision of the particular hospital department head. The second World War interrupted the progress of this plan. But before that occurred the Medical Board of the Hospital sent Dr. Arthur McGraw to consult with the State Superintendent of Instruction in Lansing. His mission was to find out what requirements had to be met before an educational institution could be given the authority to grant a degree. This was an exploration of the idea only, although the Board was convinced of the teaching abilities of the staff and of the quality of the teaching being done as well as the abundance of the material available for teaching.

It was interesting to learn that two important features were lacking in the Henry Ford Hospital. The first was adequate classrooms and physical facilities for teaching; the second, basic science laboratories manned by a qualified teaching force.

The knowledge that these two factors were lacking led to changes in the Henry Ford Hospital which are today of the greatest significance, and more and more they tend to bring very potent influences to bear upon the future.

Included in the plans of the seventeen story Clinic Building of the Hospital were the results of study of the needed teaching facilities.

The provision of an auditorium of unusual conveniences and usefulness for assemblies of size is now a feature of the Clinic Building. Throughout the seventeen stories of this building, devoted to diagnostic and therapeutic work with ambulatory patients, are larger and smaller class rooms, staff conference rooms, even specialized laboratories in addition to the clinical laboratories of the hospital. A busy daily schedule of teaching is published weekly, focused upon the men in training, from the first year interns up to and including men in advanced specialized fields. The activities of the Nurses Training School are added to those of the School of Dietetic Interns, the Schools of Medical Technologists and of X-ray Technicians, and of Physical Therapy and of Occupational Therapy, to give the environment of medical care a real atmosphere of academic medicine and teaching, in this non-university hospital.

Today, in the foreground of the Hospital activities is the challenge of graduate education. This is now an established function of the Staff, and desirable in the minds of its members, although time-consuming in their schedules. This is not to be confused with postgraduate work, interpreted as refresher courses, and similar make-up or getting-up-to-date efforts. No belittling of the value of such work for men who want it, or need it, or are inclined to provide it, is intended.

The carrying of training further into advanced phases of medicine, surgery, and the specialties represents the aim of the present active curriculum. It might be said that this principle actually begins with internships and is carried through the residencies, even though the problems of practical application, of handling of patients and of training of reflexes may seem to predominate at times.

This policy of graduate education established as it is today is invaluably undergirded by the basic sciences grouped in a building which is a laboratory, research and teaching center, located on the Hospital grounds; and intimately related to the teaching and research activities of the Hospital staff. Indeed it is an indispensable feature in a great educational institution which has already trained nearly two thousand men now located in different professional capacities throughout this and other countries.

To this Hospital, then, is added the provision of the Basic Sciences, designed as pure sciences, operating within their own fields in research, and yet so near to the members of the clinical staff and their work as to feel the prevailing interests and the answers being sought in the minds of the Hospital Staff proper. There is no question of the advantages to the clinical staff in having so near-at-hand laboratories of Biochemistry and of Physics, functioning in addition to their own particular interests, to aid and abet the researches of the clinical men, and to collaborate with them when problems are of mutual interest.

The greater goal will be attained when other basic fields are represented by departments also, and the whole Institute properly housed in its own building, operating under one head, a committee of the staff, or one individual, who will coordinate the work to its greatest productiveness and provide even better the relationship desired by the Clinical Staff. Then, hopefully, it will be able to operate under its own budget as well. These accomplishments will earn for the Edsel B. Ford Institute for Medical Research its place in the sunlight and the full recognition of the significant purpose of its origin.

Whether the present curriculum of graduate teaching becomes more formal and attains the right to award graduate degrees in specialized fields, the added requirements are now fulfilled. Medical education in the graduate field is firmly established, the required facilities are available, and the provision of groundwork science is now a reality.

EDSEL B. FORD MEMORIAL LECTURES

1952

"Experimental Production of Congenital Anomalies Through Delinquencies in the Maternal Diet", DR. HERBERT M. EVANS, Institute of Experimental Biology, University of California, Berkeley.

1953

"Some Results of the Application of Electron Microscopy to Medicine", DR. JAMES HILLIER, Research Physicist, Radio Corporation of America.

1954

"The Hormones of the Posterior Pituitary", DR. VINCENT DU VIGNEAUD, Cornell University Medical College, New York.

1955

"The Future of Enzyme Research", DR. LINUS PAULING, Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena.

1956

"Oxygen Physiology, Normal and Pathological", DR. DONALD D. VAN SLYKE, Brookhaven National Laboratory, Long Island, New York.

1957

"Molecular Genetics", DR. GEORGE GAMOW, Department of Physics, University of Colorado, Boulder.

ACKNOWLEDGMENT

The Departments of Physics and Biochemistry take pleasure in acknowledging their indebtedness to the Trustees and Officers of the Henry Ford Hospital. Without the spirit of investigation which they have fostered, their responsible management, and their extensive financial support, no program of consequence could have been undertaken.

Acknowledgments are also made to the following private and public organizations:

Adelaide Skinner Fund

American Heart Association

Michigan Chapter, Arthritis and Rheumatism Foundation

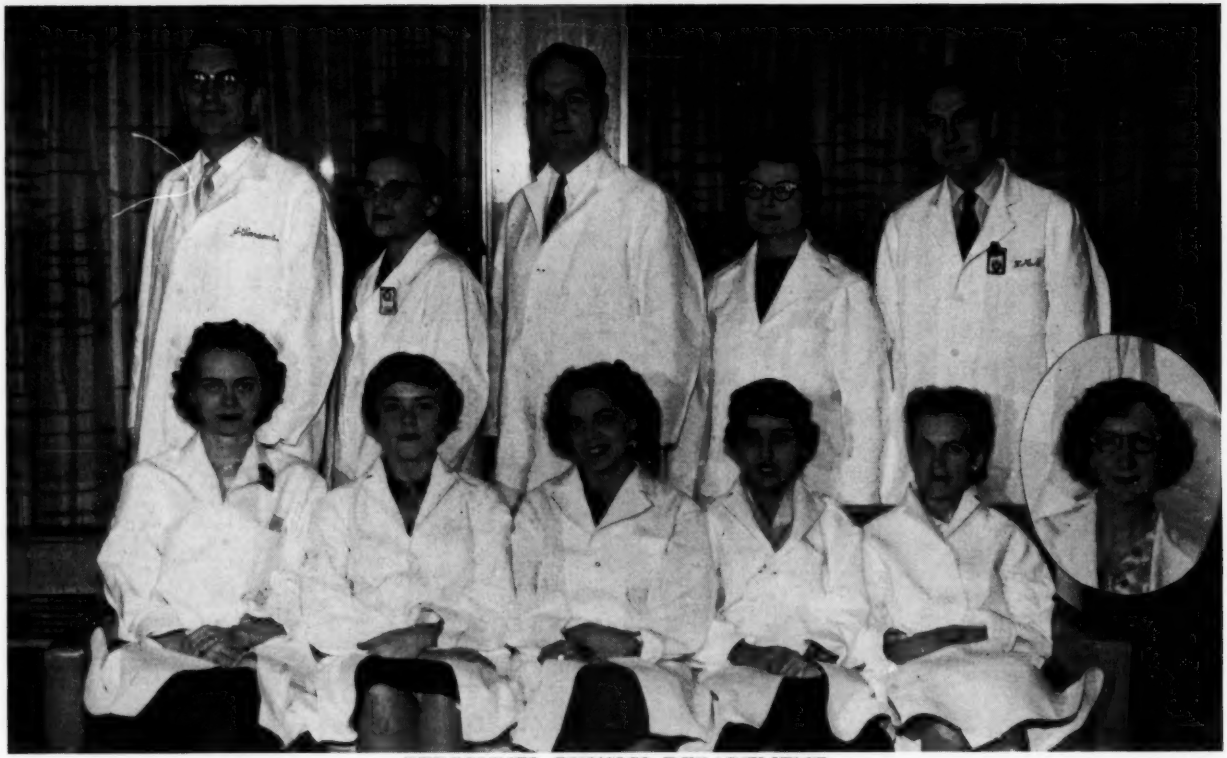
Michigan Chapter, National Nephrosis Foundation

Michigan Heart Association

National Institutes of Health, U. S. Public Health Service

Office of Naval Research, Department of the Navy

Financial support provided or assured by these sources since 1950 exceeds $250,000, and has played an essential role in development of the Biochemistry Department. Representatives of these organizations have also assisted our work by their encouragement and helpful suggestions.



PERSONNEL PHYSICS DEPARTMENT

Standing, left to right: Jonathan Parsons, Gloria Jenkins, John H. L. Watson, Catherine Alt, Luther E. Preuss. *Seated, left to right:* Juanita Velasco, Margaret Hendricks, Virginia Valentine, Dorothy Hebert, Verna Lewis. *Insert:* Lucille DuSault.

THE PHYSICS DEPARTMENT

JOHN H. L. WATSON

The Physics Department in the Edsel B. Ford Institute for Medical Research was a completely new activity in the Henry Ford Hospital community when it was first organized in May of 1947. It was set up with five original emphases in mind: three of these, radioactivity, crystal analysis, and electron microscopy were activated early and have continued to develop; the others, emission spectroscopy and electronics, are extensions for the future. To develop the three fields of radioactivity, crystal analysis and microscopy, personnel and instrumentation in the respective fields were obtained.

Luther E. Preuss, M.S., University of Wisconsin, joined the department on September 1st, 1948 as physicist in charge of radioactivity studies and several weeks later on September 20th, Jonathan Parsons, a M.A. Physics graduate of the University of Michigan, took charge of crystallographic research. As their contributions to science and the Institute developed, they were made Associates in the Department. For a period in 1950, Dr. Marcel Vanpee, Ph.D., University of Minnesota, joined the staff as a physical chemist to work on problems related to the effects upon gases of alpha ray bombardment. When Dr. Vanpee left the staff he was not replaced because of lack of suitable space. A more recent staff addition in February of 1954 was Miss Lucille DuSault as a radiation physicist to work in Radiology and Physics. Miss DuSault is a B.A. graduate from the University of California and is certified as a Radiological Physicist by the American Board of Radiology.

The Physics Department had no 'home' until sundry rooms used for storage, print shops and other pursuits on the second floor of the Service Building were redesigned by Physcis staff and set up for Physics in late 1948, thus initiating what has now become standard practice for each new research group entering the Hospital family — finding an unused corner of the Service Building and making it inhabitable as a laboratory.

It was logical and expected that the Physics Department should develop a radioactive isotope program for the Hospital and this has been done. With first beginnings in 1949 in a small but well equipped laboratory, radioactive doses are prepared and follow-up studies completed for all clinical uses of radioisotopes in the Hospital. From December 1950 until July 1st, 1957, 2246 separate doses of I-131, 212 of P-32, 118 of Au-198, and 33 of Co-60 labelled B-12 have been provided, and all of the resultant emphasis conducted by Physics Department personnel. Simultaneously a broad program of both medical and purely physical research has been conducted with radioactive materials. Institution of the radioactive program saw initial deliveries from the A.E.C. pile at Oak Ridge of about 1 millicurie per month. The present delivery rate approaches 2 curies per month — a 2.10^3 times increase. Recently a training program has been instituted wherein Physics trains 4 clinical men (chiefly radiologists) in each succeding six months period in the handling and administration of radioactive isotopes.

With limited man power and research facilities a definite policy of collaboration with Hospital and outside institutions has been followed in many researches, particularly

those involved with biology and medicine. The staff has been encouraged to follow parallel paths: (1) in problems of pure physics, within the department, with and without immediate medical aspects and (2) in collaborative efforts with research-minded clinical men which might in some cases lead to clinical overtones. Among the publications of the Physics Department one finds therefore, the results of collaborative efforts with many institutions as well as work reported purely from the Department and the Institute itself. Institutions represented in such collaborations have been Wayne State University, Chemistry Department, (3) (4) (5) (14) (91); The University of Havana School of Medicine, Department of Experimental Pathology, (17) (24) (25) (28) (42) (44); The Institute of Health and Tropical Diseases, Mexico, (25) (28) (42) (44); The University of Minnesota and The Carbide and Chemical Corporation, (33); Laval University, Chemistry Department (36); General Motors Corporation, Research Laboratories Division, (62) (77); M. W. Freeman Company, (96) (103) (117) (145) (161) and; The Laboratorio de Virus, Instituto Butantan, Brasil, (99) (158) as well as with Divisions of Henry Ford Hospital.

While in each section of the department the medical and biophysical studies have been a direct application of instruments and methods to particular clinical problems, in their purely physical researches the projects have been chosen generally for their emphasis on the properties and structure of matter and of the solid state in particular.

The usefulness of x-ray diffraction in identification of unknowns, widely recognized in industry, has been slower to develop in biology and medicine. This laboratory has made noteworthy advances in this subject and Mr. Parson's contributions have been recognized by his appointment to the A.S.T.M. and A.C.A. Joint Committee on Chemical Analysis by Powder Diffraction Methods. In the x-ray diffraction laboratory Jonathan Parsons has been developing new analytical techniques and instrumentation (23) (93) (113) for the identification and structural description of organic and biological compounds (73) (84) (106), particularly of the steroids with William T. Beher of the Biochemistry Department (107) (108) (123) (153) (157) and as a complementary procedure with electron microscopy (99) (158). Clinically, it has been clearly shown that for convenience and accuracy, identification by x-ray diffraction of body concretions is often preferred to chemical methods, (59) (75) (127). A large number of urinary calculi are examined each year as a service to Urology and in order to add to the body of statistical data being collected on these materials.

Although the early years in the radioactive laboratory were used mostly in the clinical development of radioactive materials in the Hospital, research in physics and in medicine, followed quickly. Luther Preuss has developed numerous devices, and laboratory techniques for appropriate handling of radio active materials (13) (27) (54) (64) (66) (67) (68) (82) (128) (130) (131) (132) (133) (136) (142) (146) (147) (155) (162) (163). His researches on the propagation of metal evaporates, using radioactive isotopes of metals (78) (137), and his later adaptations of these techniques to pin-hole autoradiography of high temperature sources (164) have marked unique advances in vacuum and evaporation research. Much of his work has been included in recent treatises on high vacuum, including "High Vacuum Techniques"

by L. Holland in this country and the German publication "Vakuum" overseas. Mr. Preuss is co-chairman of the Michigan Nucleonic Discussion Group.

The electron microscopy laboratory has applied itself to the pure sciences of physics, chemistry and biology rather than to clinical researches. Its contributions have been recognized here and abroad and the laboratory has continued to contribute successfully to the scientific literature and meetings. Some of the contributions to the science, electron microscopy itself, have been related to specimen preparation (152), micrograph interpretation (8) (46) and to the philosophy of the subject (2) (37). The first applications of cinematographic methods to the electron microscope were done on this continent in this laboratory (30) (31). The dangers of x-radiation incident to the use of the microscope were studied here (38) (89). Reports were issued on the effects of the electron beam upon specimens (9) (10), and the calibration of electron microscopes by internal standards was investigated (62) (77).

In chemistry and crystallographic studies, maximum use of the electron microscope has been made in the field of colloidal crystals (3) (4) (5) (14) (96) (99) (103) (117) (158) (161) and of colloidal solutions (1) (20) (48) (91). The first micrographs of the solid and liquid products from alpha ray bombardment of CO,C_2H_2 and CH_2CHCl were taken (7) (33) (40). The first crystals reported from such reactions were announced in 1955 (95). The morphology of the cuprene polymer was studied and detailed (36).

In biology the microscope has been used to study chick embryo erythrocytes (17), isolated single nuclei from laboratory animals (24) before the days of thin sectioning (118), artifacts resembling virus particles (25), stromatolysis in human erythrocytes (28), flagellation in species of Genus, *Treponema* Schaudinn, treponemas of certain tropical diseases of Yaws and Pinta (44), and crystals from gastric secretion (56). The first electron micrographs of replicas of human kidney stones (cystine stones) were obtained in 1956 (115) (122).

In solid state physics, in addition to many of the results already noted which bridge the fields of chemistry and biology with physics, recent work has seen the determination of crystalline dendritic structure in colloidal dendrites of alpha iron, determination of relations which exist between their particle shapes and sizes and their intrinsic coercive force (145) and quality control of the shapes and sizes necessary to exploitation of these alpha iron crystals in important medical applications to follow. The first direct pictures of colloidal iron particles aligned in magnetic fields, with all that such evidences mean eventually in the proper understanding of fine particle magnetics, were taken in this laboratory.

Contiguous with this scientific activity in electron microscopy have been contributions of time and effort to the administration of the Electron Microscope Society of America. The writer is the present President of the Society, serves as its Employment Referral Bureau Officer and was a Councillor for three years. Mr. Preuss has been the Society's chairman of Scientific Exhibits for 1956 and 1957.

Most of the collaborative efforts of the department with the Henry Ford Hospital have been in the field of radioactivity. Some of these have involved "Iodine Metabolism in Cases of Total Thyroidectomy" with General Surgery, "Radioiodine Tracer Studies

on Children" with Pediatrics, "Radioiodine Tracer Studies on Selected Angina Pectoris Cases" with Cardiology, "Study of Peripheral Vascular Disease by Use of Radioisotopes" with the Department of Surgery, "The 72 Hour Specific Activity of the Protein Bound Iodine as a Test of Thyroid Function" with Endocrinology and the Laboratories, "Test Tolerance to Radioactive Colloidal Gold" with Gynecology and Radiology, "Determinations of the Minimal Effective Dose of T.S.H. for the Stimulation of the Normal Thyroid Gland" with Endocrinology, "Blood Volume Studies with Radioactive Chromium-51" with Anesthesiology and Medicine. These projects have resulted in several collaborative reports, (50) (80) (112) (139) (140) (141). In addition, Physics has spent time with Neurosurgery studying methods of detecting brain tumors using I-131 labelled diiodofluorescein, and with Hematology in the utilization of Co-60 labelled Vitamin B-12 in certain blood disorders.

The Department of Physcis in the Institute has remained in close liaison with the Hospital not only in research but in committee work for the administration. The Head of the Department has served on the Medical Board of the Henry Ford Hospital, has been secretary of the Research Committee since 1953, chairman of the local Isotope Committee and chairman of the Liaison Committee for Cooperation in medical research between the Scientific Laboratory of the Ford Motor Company and the Henry Ford Hospital, and a member also of the Publications Committee.

X-RAY DIFFRACTION ANALYSIS

JONATHAN PARSONS

For many years the tools and techniques of x-ray diffraction have been used in minerology and metallurgy to determine the crystalline nature of solids. The fact that this method of analysis is non-destructive and uses only small amounts of sample makes it suitable where other, more conventional methods are not practical. This laboratory was one of the first to apply these techniques to medical and biochemical problems (23) (73) (84) (93) (113).

X-RAY DIFFRACTION METHOD. The great majority of all solids are crystalline with their constituent atoms arranged in repeating, symmetrical, three dimensional arrays. Gases, liquids and some solids (glass, plastic, tar, carbon black, etc.) may have little or no symmetrical atomic arrangement and are said to be amorphous. It is a rare event when two different crystalline substances are found to contain atomic spacings or arrangement which are at all similar.

A finely collimated beam of soft monochromatic x-rays, directed against a small sample of powdered crystal is dispersed (diffracted) in a specific manner by the particular atomic geometry inherent to the specimen, and patterns produced upon photographic film by the diffracted x-rays are characteristic of the sample. The pattern might be referred to as a 'finger print' for the unknown substance. X-ray diffraction standard data are available for many compounds and provide a physical method by which solid materials may be quickly and non-destructively analysed.

THE STEROIDS. The steroids are a family of substances critically important to animal life. Metabolic studies relating to the members of this family are being pursued by W. T. Beher, and his co-workers. Since these organic compounds are crystalline and are present biologically in very small quantities, x-ray diffraction becomes a logical method for their analysis and identification.

With the collaboration of Dr. Beher x-ray diffraction powder data for 139 of the most biologically common steroids have been obtained. The obtaining of these standard data for the steroids has been a necessary step in contemplation of the further use of the technique for the chemical analysis of biological extracts. To make the data available to other investigators in pharmaceutical, medical, and research institutions, powder photographs and pattern data from the studies have been reported in a series of four papers which have appeared in *Analytical Chemistry* (107) (108) (123) (153). These data have been correlated with melting point determinations for each steroid. Since two or more polymorphic crystalline forms often may exist for some steroids, information about the solvent and method of crystallization are also reported. An index to the 3 strongest diffraction lines for each steroid pattern is being compiled for use in identifying patterns from unknown steroids. Such an index will shorten the time necessary in searching for a matching standard pattern. This data will be included in the x-ray powder data file published jointly by the American Society for Testing Materials and the American Crystallographic Association.

The usual method of extracting the 3β-hydroxy sterols is to precipitate their digitonides. Comparative infrared spectrophotometric and x-ray diffraction studies,

have been done with O. H. Gaebler, W. T. Beher and G. D. Baker (106) (157) for six of the common steroids and their digitonides to determine the conditions under which each method of analysis is most sensitive. The use of x-ray diffraction definitely establishes sterol digitonides as chemical compounds, whereas, infrared specrophotometry does not distinguish between digitonin, a simple steroid-digitonin mixture, and the corresponding sterol digitonide.

The success of the x-ray diffraction methods for the analysis of the steroids is an encouragement to extend these studies to include other classes of biological compounds, the lipids and fatty acids.

URINARY CALCULI. An accurate knowledge of the structure and chemical composition of urinary calculi is needed in attempts to find a solution to the problem of the etiology and prevention of kidney stone formation. The obvious similarity between urinary calculi and minerals suggested the application of x-ray diffraction techniques in studying their crystalline components.

The inadequacies of chemical analysis for urinary calculi are rather widely known. These include: 1. Confusion as to the nature of the reactions which take place in qualitative tests. 2. The minute amount of sample often available may be insufficient for good qualitative tests. 3. The mixture of organic substance in these stones may invalidate the chemical tests. Since the patterns of x-ray diffraction emanate from the structural differences of the crystalline material, this method is not subject to the above inadequacies. A fraction of a milligram of powder is all that is required to obtain a diffraction pattern.

The following list contains the compounds which have been found in an examination of 361 urinary calculi. The percentage occurrence of the particular molecules, or combinations of them, in the population of the 361 stones is given in the table.

	%
Calcium Oxalate (pure)	50.97
Calcium Oxalate plus Apatite*	10.80
Apatite (pure)	9.42
Uric Acid	9.00
Magnesium Ammonium Phosphate (pure) (127)	6.37
Mag. Amm. Phosphate and Apatite plus Calcium Oxalate	3.00
Mag. Amm. Phosphate and Apatite	1.94
Calcium Hydrogen Phosphate	1.39
Cystine (59)	1.39
Zinc Phosphate (75)	1.11
Sodium Acid Urate	0.56
Other combinations not listed above	4.05

*Apatite, basic calcium phosphate.

MICROSCOPIC PATHOLOGICAL MATERIALS. The pathologist often finds minute crystals imbedded in tissue sections which have been prepared for microscopic examination either from biopsy or autopsy. The specific identification of such crystals by conventional means is usually very difficult due to their minute sizes. The x-ray diffraction section of the Physics Department has frequently been able to identify these crystals by x-ray diffraction.

Two mounting techniques are employed depending upon the abundance of the tiny crystals. A direct, needle-like slice of the tissue, mounted in a powder diffraction camera will show the pattern of the crystalline material if the crystal concentration is great enough. Such needle-like slices of tissue are inserted into small thin-walled tubes made of amorphous glass of low x-ray absorption coefficient. If an ignited or ashed sample produces a good x-ray diffraction powder pattern this first method of mounting also may give a pattern. The ashed sample pattern itself cannot be used as a final judge of composition due to the chemical changes which usually occur during the ignition process. A second method of x-ray diffraction study is used when only a few microcrystals are present. This consists of using a special micro-camera which permits proper alignment upon a microscope stage, to allow a small cylindrical x-ray beam to traverse the particular microcrystal in question.

Such x-ray diffraction analysis procedures upon biological microcrystals have been used to identify talc (magnesium silicate) in heart pericardial tissue, calcium phosphate in broncholiths, mercuric sulphide in a tattoo granuloma, thorium dioxide in spleen, and sodium acid urate in an ear tophus.

MICRORADIOGRAPHY. The nature of the radiation useful in x-ray diffraction is also suitable for x-ray microradiography. Here a small specimen is placed in contact with an extremely fine grain photographic emulsion. When soft radiation is allowed to fall upon such a specimen a miniature radiograph is produced which can be subsequently enlarged photographically.

A technique for injecting a suitable radiopaque fluid into the vascular systems of experimental animals was developed in this department in 1951. The vascular systems of organs were studied, by placing relatively thick sections of tissue, averaging 100 microns in thickness, into direct contact with a fine grain photographic plate. By making two successive x-ray beam exposures, plates were obtained which could be viewed upon a stereoscope. By this method very fine internal vascular details of normal and pathological nature can be detected to a degree not possible in a photomicrograph.

Recent improvements in the design of x-ray source units for microradiography have obviated the necessity for vascular injection in order to get suitable contrast in contact microradiographs. With the acquisition of such a unit containing a 0.3 mm x-ray tube focal spot and 50 micron beryllium tube window, it is planned to make microradiographs of thinner tissue sections as well as other materials which possess varying internal densities not seen by surface photomicrographs.

SUMMARY. X-ray crystal analysis is currently being applied in a wide range of investigation on colloidal crystals of various types (95) (99) (117) (158), barbiturates

and snake venoms. The writer is a member of the Joint Committee on Chemical Analysis by Powder Diffraction Methods. This group is sponsored jointly by the American Crystallographic Association and the American Society for Testing Materials. Its purpose is to promote the use of x-ray powder diffraction by abstracting from the literature x-ray diffraction standard data as the latter is published. Word has been received that the powder diffraction data for the steroids obtained in this department are now being processed for inclusion in the next issue of the X-ray Powder Data File and its index volume.

PHYSICS IN RADIATION

LUCILLE DUSAULT

In addition to experience as a physicist in the problems of radiology involving the patient, (problems of health physics, treatment planning, dosage calibration and mapping, and the like), the writer brought to Henry Ford Hospital a deep interest in research on the subject of time-dose relationships, i.e. the adjustments, which must be made in a dose of radiation to compensate for a change in the time during which the radiation is administered in order that the effect of the treatment will be the same. As a co-author, two papers had been published previously, one giving the time-dose curve for cancerocidal effect upon squamous cell carcinoma of the cervix uteri* and the other for adenocarcinoma of the corpus uteri**. After coming to Henry Ford Hospital the general subject of time-dose relationships was studied further and was discussed by the author in a paper before the American Radium Society in 1954***.

The possibility of improving the results of radiation therapy by a change in the fractionation of the total dose was suggested at that time. In order to explore this, a project was begun in the Fall of 1955 with Dr. Iler. This is partly supported by a grant from the American Cancer Society. Successive groups of C3H mice with spontaneous mammary adenocarcinomas were given increasingly larger doses in the same overall time, but with different spacings of the individual treatments within that time, in order to find a combination of dose and interval between treatments which would show better results than other combinations. A statistically significant difference in results between two of the combinations has been noted and will be reported shortly.

Another subject under investigation is the problem of bolus for telecobalt therapy. Ordinary bolus, as used in conventional x-ray therapy, cannot be used because of the production of electron equilibrium at the surface of the skin with consequent loss of the skin-sparing aspect of cobalt therapy — one of its chief advantages. Consequently, a tin-lined bolus was tried, the optimum thickness of tin determined, and resultant build-up under the skin demonstrated. The same idea can be applied to treatment cones.

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*Optimum Dosage Studies for Radiation Therapy of Carcinoma of the Uterine Cervix. *Radiology, 62*, 862 (1954) (with James F. Nolan, M.D.).

**Radiation Treatment of Carcinoma of the Corpus Uteri. *American Journal of Roentgenology, Radium Therapy, and Nuclear Medicine, 71*, 669 (1954) (with W. E. Costolow, M.D., James F. Nolan, M.D., and G. C. Budenz, M.D.).

***Time-Dose Relationships. *American Journal of Roentgenology, Radium Therapy, and Nuclear Medicine, 75*, 597 (1956).

THE RADIOACTIVE ISOTOPE RESEARCH PROGRAM AND FACILITY

LUTHER E. PREUSS

FUNDAMENTAL PHYSICAL RESEARCH

THIN FILMS: The most productive and stimulating facet of radiotracer usage is its application to special problems where the more conventional methodology may have failed. For example, the author's research on vacuum distillation phenomena and resultant condensed films, combined with electron microscopy of the structure of such films, has been strongly aided by the utilization of the tracer technic. Unsuspected, productive research avenues have been opened in this work by the tracer method. The distillation ray camera and the crucible dispersion studies, described in the following are examples.

Autoradiography, a prime investigative tool, has proved invaluable in the study of both the macro- and micro-array of *very thin* radioactive metal layers. Fundamental investigations of the chemographic mechanism (87), which stemmed from the 1950 A.E.C. course on advanced autoradiography*, have been begun. A related series of experiments is in progress to clarify the role of the Russell Effect† with newly condensed metal distillands. The sensitivity of the technic has been extended in this laboratory to surface concentrations of the order of 10^{-9} grams per cm^2.

A fundamental technique has been developed for the calculation of tenuous film thickness utilizing Cr^{51}, Au^{198}, and W^{185}, as well as other isotopes. Comparisons by standardization give accurate assay of mass per unit area. Sensitivity has been achieved which is 10^5 times better than that obtainable from multiple beam interferometry. With the assumption of a coherent layer, metal films 10^{-5} angstrom units thick have been measured (78).

The same system has been applied also to the investigation of concurrent distillation rates for pure materials in mixtures and alloys for varied applications of Raoult's law. This is accomplished by a special scheme using multiple distillations and the solution of simultaneous equations in the assay formulae, with double radiation detectors of varied sensitiivties. It has been shown‡ that concurrent distillation between the refractory filament source material and the charge occurs in an unsuspected high ratio. This phenomenon may have some effect upon the structure of thin films, such as are used for shadow casting in electron microscopy.

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*Acknowledgment is made to Dr. G. Boyd, Director of the A.E.C. course, for extended council and aid.

†The chemographic effect attributable to oxidation phenomena at the interface of newly prepared metallic surfaces

‡Proceedings of the 1956 National Symposium on Vacuum Technology.

Paralleling this, vacuum evaporation dispersion studies for radioactive charge material were carried out as part of a general program involving phenomena associated with the vacuum shadowing process. Unique tracer work (78) has demonstrated the intrinsic sensitivity and has proved that the assumption of symmetrical, spherical dispersion made by many workers is erroneous and may lead to errors of the order of -75% to $+1000\%$ in the prediction of film thickness.

A segment of this interest in ultra-thin radioactive layers of metal, has produced a new approach to the study of macro-and mirco-surface detail. This consists of the production of a thin radioactive deposit by evaporation upon the surface in question, its subsequent autoradiographic exposure, and reproduction of the film's array. The method has demonstrated resolution of structures down to 40 microns in preliminary studies, and the order of 20 microns under special conditions in continuing work (86).

A BETA AND GAMMA-RAY VACUUM CAMERA: An outgrowth of the interest in thin radioactive film deposition morphology is the design, construction, and application of a beta-ray camera. This involves an investigation of image formation by the autoradiographic adaptation of the simple pin-hole camera†. The source configuration of very highly radioactive specimens may be delineated by this process. Fig. 1 is a negative of an autoradiogram taken by this system. This is an image of an extremely concentrated source of radioactive P32, laid down in a peripheral deposit. This vacuum camera system has proved highly superior to the gross apposition autoradiography usually used. This work is being extended to such a camera design for gamma ray emitters, in which the aperturing system is much more heavily shielded.

THE BETA-EXCITED APPOSITION AND POINT X-RAY SOURCE: Two new types of x-radaition sources are presently under study in this laboratory. The first is a planar design for apposition use with interchangeable targets and with variable beta-source configurations, Fig. 2. The second is a source-target mixture design, wherein the goal is a "point source". Both utilize the impingement phenomena of beta radiation from the radioactive nuclei of certain synthetic radioisotopes. Some of the pure beta emitters used for this interaction are Sr^{90}-Y^{90} mixtures, P^{32}, Pm^{147}, W^{185}, Tm^{170} Ca^{45} and Ti^{204}. Control of the bremsstrahlung ratio and solution of the geometrical design problems offer two exceptionally stimulating challenges†. The interrelation between beta energy, source intensity, efficiency, target thickness, radiation shielding, and geometry is a somewhat unexplored field; the best geometrical configuration for a specific photon output is not well established. Experimental work is in progress to ascertain the spectral characteristics of varied sources and to establish the quality and output intensity by radiographic means. The efficiency of the fluorescence principle and the characteristics of transmission and reflection targets is of some concern in this study.

Preliminary resolution studies have been made with the planar apposition source. These were carried out with separate source-target geometries using P^{32} as source

†Manuscript in preparation.

with a Cu target. Resolution on 200 mesh nickel grids is of the order of 75 microns and is presently being improved by the use of more favorable source-target geometries. Homogeneous target-source mixtures with P^{32} betas have been made with diameters as small as 3 mm. With a precipitated silver salt target these have produced radiation of some reasonable radiographic quality. Total source activity has been kept low for health-physics reasons and exposure periods are relatively lengthy.

An illustration of the planar apposition source design is given in Fig. 3. This is a cylindrical brass housing, one inch in diameter and height. The planar, disk-like source is seated in the housing top above a reflector target. The transmission target is fixed above and in contact with the P^{32} source, to form a target-source lamination. This '*backing target principle*' is used to improve the x-ray production efficiency and flux. Characteristic and "white" radiation are both produced in the double target and expedite the autoradiographic exposure when the source is placed in apposition with the proper emulsion. Fig. 4 is an illustration of the radiographic quality obtainable with a P^{32}-Ag, source-target mixture. The subject is a miniature electronic tube. Silver was precipitated as the phosphate to form the homogeneous target arrangement. Kodak *special* Royal Blue emulsion was used with DuPont Lightening intensifiers†

THE BETA RAY PROJECTION MICROSCOPE. This investigation has grown out of this author's interest in identification of surface and internal structure through a technique of projection and apposition macro-radiography. This utilizes directly the particulate radiation from selected isotopic agents and differs thereby from the simple apposition study. The key to the experimental design is that in certain high specific activity materials the requisite activity may be concentrated in a very small total volume. This constitutes essentially a point source and serves in this projection macro radiography as the 'illumination' for certain specimens which are held in apposition to the radiographic emulsion. The study has used the radiation from pure beta and gamma emitters as well as from mixed sources and from electron conversion transitions.

VACUUM DISTILLATION CAMERA. One of the more intriguing problems has been that of the physical mechanism of metal vacuum distillation. Various questions related to geometry, fill-in, creep, scattering reflection, etc., had been left largely unanswered in the foregoing studies, but a new method has been developed by which many of these problems have been uniquely resolved. This involves a combination of distillation camera exposures and autoradiography which provides a true image of the distillation source at the instant of molecular distillation. The resultant autoradiographic image gives a quantitative representation of the real region from which the distilling atoms emanate. The theoretical limits of sensitivity of this technique are as low as 10^{-5} grams of total charge distilled and 10^{-7} grams of image condensate.

Fig. 5 is a reproduction of an autoradiogram made for the distillation of radioactive gold from a helical tungsten wire crucible. This image represents the true distillation volume beyond which evaporating metal atoms travel in collision-free paths.

†Manuscript in preparation.

This is an end-on view by the distillation camera, with the optic axis coincident with the axis of the helix. Note, that the turns of the helix are clearly demonstrated. Fig. 6 is a distillation camera exposure of the evaporation characteristics of a radioactive W^{185} bar crucible. WO_3 on the 'hot' radioactive bar has diffused to the vertical supports which also serve as a source of W^{185} O_3.

ACKNOWLEDGMENT. The radioactive isotope laboratory staff is indebted in its physical research program to the Phoenix Project, Isotope Laboratory, Reactor Facility Building, North Campus, University of Michigan for aid and consultation and for use of facilities. We are particularly grateful for the cooperation of H. Gomberg and E. Emmons of the Phoenix Project.

BIOPHYSICAL RESEARCH AND CLINICAL PROGRAM

BIOPHYSICAL RESEARCH. Many collaborative researches involving the nuclides; P^{32}, I^{131}, Au^{198} and Co^{60}, have been undertaken with clinical divisions of the Henry Ford Hospital. In addition to projects mentioned elsewhere in this report, such biophysical studies have included investigations of phenomena related to, *I^{131} serial uptakes, Au^{198} in transport and therapy, radio-colloids for interstitial injection, erythrocyte survival, and electrolyte disappearance rates.* These nuclides have also been used regularly in clinical tracer and therapeutic studies. The methodology of both the physical and related biophysical systems in all such work is not well established and consequently appreciable time is spent in fundamental studies of tracer systems. For example, recent investigations having to do with the stabilization of colloidal suspensions (136) and effects of geometry on tracer systems (54) have been reported from this laboratory and have yielded more accurate assay methods.

DOSIMETRY AND HEALTH PHYSICS. Each nuclide and activity level may require special devices for adequate assay and use. The radioactive laboratory has had a continuing duty to adapt and develop custom instrumentation without which satisfactory tracer operation is impossible.

Dosimetry investigations, which have been centered chiefly on low energy gamma and x-ray flux rate measurements, have resulted in the development of a unique null-method electrometer*. This has made accurate dosage rate determinations feasible at exceedingly low dosage flux with certain ionization chamber designs originating in this laboratory. The importance of being able to evaluate personnel radiation hazards properly was recognized early. This laboratorie's active interest in all the standards and methods of health physics is reflected in the fact that laboratory dosage remains well below 5% of the recently lowered tolerance levels (155).

*Manuscript in preparation.

LABORATORY CONCEPT AND IMPLEMENTATION. A formal isotope program is accompanied by unique problems of design and construction of laboratories and equipment (27). Development studies here have included those of architectural design, shielding, air conditioning (131), etc. The isotope laboratory was built for a triple phase research and development usage: A, for tracer and radiation research within the Physics Department itself; B, for research carried on intramurally by and with other departments of the Institute; C, and for extramural research conducted with Hospital divisions. In addition, the laboratory was intended as a developmental center for certain tracer techniques to be applied clinically in the Henry Ford Hospital. The diversified nature of the proposed uses for the laboratory made its design and operation a stimulating task.

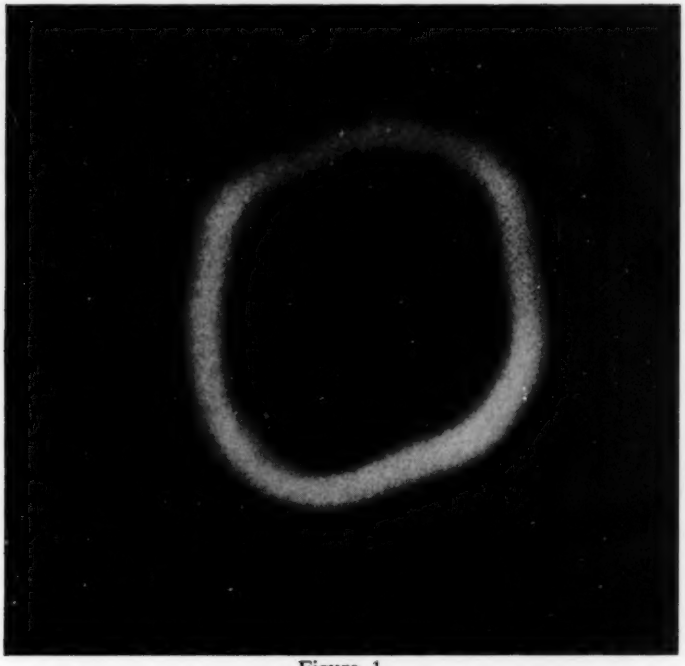

Figure 1

A negative of beta-ray camera exposure. Bright areas correspond to regions of high intensity beta emission. This is a characteristic peripheral deposit of concentrated P^{32}. System pressures, 200 u Hg^{-1}.

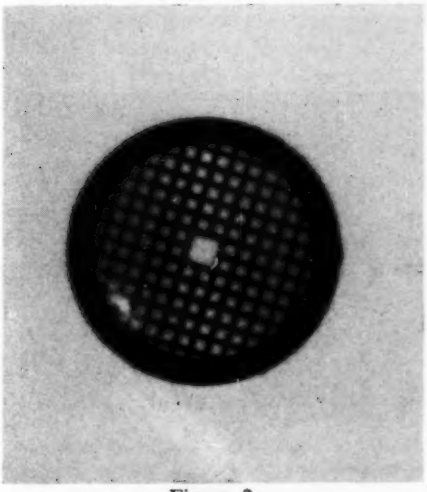

Figure 2

A resolution study on a 0.125 inch copper grid by the apposition method. A planar source of radioactive Cr^{51} is the source of radiation.

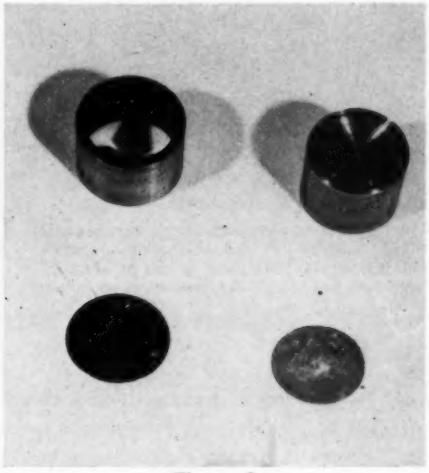

Figure 3

The planar P^{32}-Cu source-target design is shown disassembled. The entire radiation source exclusive of shielding occupies less than one cubic inch.

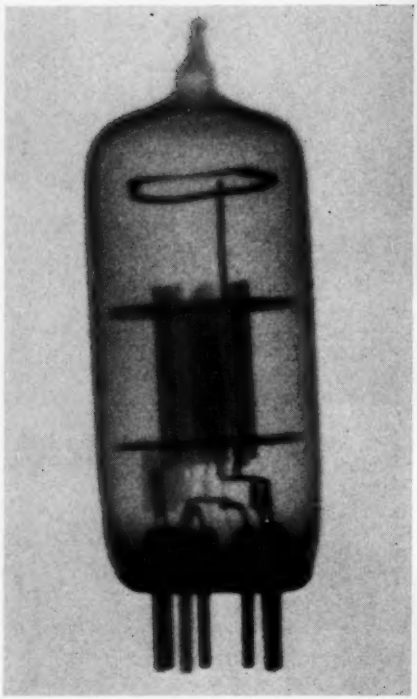

Figure 4

The P^{32}-Au intimate source-target mixture produced radiation of this radiographic quality. The source was less than 3 mm in diameter. Note the fine detail despite the short source distance of 15 cm.

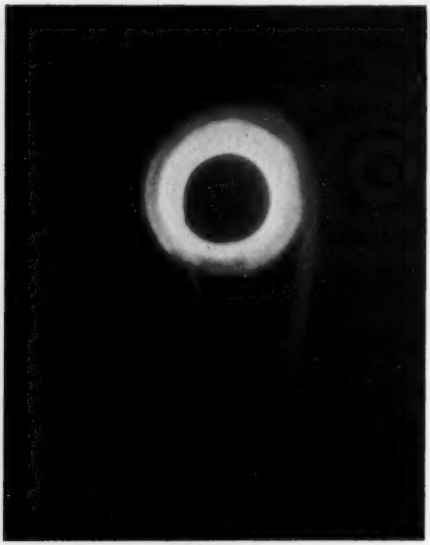

Figure 5

A distillation camera exposure was made of the evaporation sequence of radioactive Au^{198} from a tungsten helix. This is a negative of the autoradiogram. Exposures were on Kodak MLS and development was in D-72 at 1 to 1. Note that the regions of distillation of the gold are clearly identified with the outline of the tungsten helix.

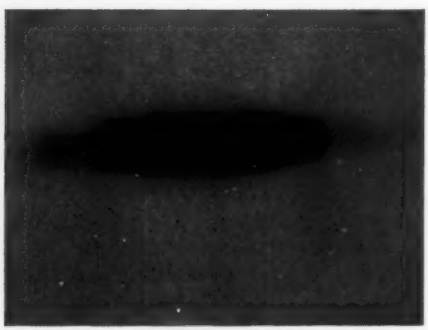

Figure 6

A horizontal tungsten bar tagged with W^{185} has been oxidized and then vacuum distilled before a distillation camera. This is a positive of the autoradiogram made on the Kodak MLS. Dark areas correspond to radiation-induced latent image formation. The distillation characteristics of the short horizontal bar are clearly defined.

ELECTRON MICROSCOPY, IRON CRYSTALS AND MEDICINE

JOHN H. L. WATSON

In electron microscopy one deals with objects and structures which have infinitely small dimensions, but which must be comprehended with a finite human mind. It is difficult for most of us to conceive of the ultimate "small" or the ultimate "big", and yet we use terms like "atomic" or "nuclear" quite freely. No one has ever really measured the diameter of an atom but only its mean free path. The atom is a nervous part of creation, it cannot stand still long enough to be measured. Still, we say that the diameter of an iron atom is 1.26 angstrom units†, meaning that it is most probable that the influence of the iron atom will be felt, more or less over this distance. In electron microscopy too we deal with such linear units approaching atomic dimensions.

For example in the discussion to follow, iron crystals will be considered which are anywhere from 100 to 5000 angstrom units thick and may be from 100 A.U. to many microns long. A single 30 micron side cube of cement dust could hold a billion of the iron crystals. Now, there are two classes of people who deal in billions easily; those who handle our national budget, and astronomers, but it is difficult for the average person to visualize a billion without certain mental stimulation. For example, if one billion seconds were counted using a clock, it would take 32 years to count a billion seconds, counting day and night without stopping, (with the standard 8 hour day the job could not be completed in a human life time): or if one stacked one dollar bill upon another (regardless of inflation), a billion-dollar pile would be 57 miles high (each bill being about 0.0036 inches thick). So a billion is a rather large number and should be treated with respect both to its own significance and the advantages to be gained in biology, medicine, magnetics and metallurgy by the use of single iron crystals as fine as tobacco smoke.

Many important basic contributions from electron microscopy have emanated from Institute projects. It is not possible nor intended to describe all of these here but a representative subject has been chosen which has certain novelty and is as yet not completely reported elsewhere. The discussion will center about what the electron microscope and related techniques have told us about the structure and properties of colloidal crystals of alpha iron. This is a fairly recent, in-progress emphasis which, because of its effects on the study of fine particle magnetics and because of the potential of its medical uses, may represent a considerable contribution to both physics and biophysics.

STRUCTURE. Electron microscopic studies of certain colloidal alpha iron crystals made in an electrolytic process were undertaken because of a long standing, laboratory interest in colloidal crystals and because of important inherent medical applications. Before the medical uses could be realized, certain basic properties of, and phenomena

†One centimeter $= 10^4$ microns (u) $= 10^8$ angstrom units (A.U.).

related to, the tiny crystals had to be known and determinable in advance.

Many of the solid state properties of the material have now been established and a new tool for medical research and application is available. Although iron itself is allotropic, these crystalline particles are always *alpha* iron (96) which has a body centered cubic structure. The process of manufacture is controllable and continuous and reproducible as far as size and shape of the colloidal crystals are concerned. It has been shown that their size and shape are not only functions of the conditions of formation but of the inherent crystalline properties as well. In turn, unique physical properties and consequent applications of the crystals are a result of morphology, size and other properties. There are several crystal types, notated for convenience as, *discrete particles, needles, nodular rods* and *weak* or *strong dendrites.* From these investigations all appear to be single crystals, and the genesis of each is a dendritic growth. The single difference between the particle types seems to lie in the extent and quality of this dendriticity.

Because of the intrinsic dendritic nature of these iron particles, any complete understanding of their magnetic (145) or metallurgical properties (161) or of their medical uses will depend to some extent upon knowledge of their dendritic structure *per se*. This work represents one of the extremely few attempts to study the fine structure of submicroscopical dendrites by any method.

With techniques of electron stereo-microscopy* applied to partially etched, carbon mounted particulates (152) it has been possible to define the structures fairly completely, down to 50 and 100 A.U. The ultimate crystallites *(discrete particles)* of which the dendrite is composed appeared to be single 100 to 500 A.U. rhombic dodecahedrons as represented in Fig. 1. A monolithic linear arrangement of these, attached corner-to-corner along the cubic diagonals with the *111* planes perpendicular to the direction of dendritic growth comprises what is referred to as a *needle*. Whenever crystallites develop, in a similar fashion at periodic nodes along the sides of such a needle, a *nodular rod* is formed. Further development of the main stem and the side arms produces a *weak* or a *strong dendrite* depending on the extent of the growth.

It has been observed that three secondaries form at any node along the primary. These have 120 degrees of angle between them and each slants at about 60 degrees toward the direction of primary growth. Where tertiary arms occur they follow the same habit. The dendrite as a whole is itself a single crystal of alpha iron with certain preferred directions iron-filled. A further development, called a *filled-in dendrite,* occurs when conditions are such that the branches and crystallites "coalesce" in extended development. The weak dendrites are formed under conditions where some secondaries are aborted or lack uniformity of length and distribution along their primary. Fig. 2 is a typical electron micrograph from which these observations were drawn. This is a partially etched, carbon replica of a field of strong dendrites. Unetched, all branches were opaque, and overlapping themselves or the primary, one set always went undetected by other mounting methods. The dendrite at *A* is lying

*John H. L. Watson. *Can. J. Res., 21,* 89 (1943).

with two sets of transparent etched secondaries slanting down into the plane away from the viewer. The relatively unetched opaque series of triangulations along the primary represents a third set of secondary branches growing toward the viewer from the plane. At *B* and *C* are other dendrites oriented differently with one transparent set parallel to the plane, and a second set slanting down into it. An opaque third set, superimposed on these, slants upward toward the viewer.

A corner-to-corner (along the diagonals) development of the arms has been indicated by direct observation in many of the better resolved micrographs. In addition such growth is inferred from the observation of a 60 degree angle between secondaries and primary, as well as from the identification of sets of three secondaries with 120 degrees between them. Briefly, from electron microscopic studies a dendrite model has been established† which satisfies not only all of the microscopy observations but fulfills certain concepts concerning the crystallization of body centered metals as well. Although the dendrites tend to assume their most stable positions, Fig. 2, and do not easily demonstrate their three secondaries, micrographs of "end-on" dendrites, Fig. 3, are obtained occasionally.

An additional particulate crystal form is referred to as a *platelet* and has often been seen in these samples, Fig. 4. Many of these colloidal platelets are dendritic in two dimensions. By selected area electron diffraction these crystals demonstrate the Kikuchi N pattern with Kikuchi lines for platelets 80 A.U. or less thick and are also identified as alpha iron, Fig. 5.

MAGNETIC PROPERTIES. The magnetic properties of colloidal iron crystals, particularly in this era of the magnetic tape, the powerful miniature magnets, the transistors, and the printed circuit, are of immense importance. It has been essential to correlate shape, structure and size of these colloidal alpha iron crystals with their magnetic properties. From statistical areal analysis performed upon electron micrographs, it has been possible to conclude (a) that nodular rods and needles, Fig. 6, are those particles which contribute to the higher coercive force and that the other particulate types tend to lower it, and (b) that in some manner, as yet undetermined, the coercive force is improved as the particle dimensions are reduced to some optimum size.

Experimental evidence has shown that these crystals have coercive force of unusual magnitude. The electron microscope was able to explain the enhanced coercive force by reasons inherent in the size, shape and structure of the particles, chiefly their shape anisotropy and their monolithicity.

The processes of alignment associated with these particles has been studied by a unique method which involves suspending them in capsules of n-butyl methacrylate monomer within the region of a magnetic field. Under these circumstances the particles, affected by the field, attempted to align themselves in preferred directions

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†This model was first discussed before the American Crystallographic Association June 11, 1956 at French Lick, Indiana in the paper "The Ultrafine Structure of Elongated, Magnetic Single-Domains and of Dendrites of Alpha Iron". John H. L. Watson, Michael W. Freeman, and Nicholas N. Solimene.

against the mechanical forces of the liquid. Although certain evidences of orientation and alignment were seen, especially in the macroscopic, in the microscopic they were observed to form closed networks quickly in order to minimize their magnetostatic energies. The result was that at fields as high as 25,000 gauss, while the iron particulates appeared to the eye to be aligned by the field, in the electron microscope very little alignment of the individual microscopic crystals had actually occurred. This in itself was an important negative observation which indicated that improved, different methods of alignment would be needed.

In order to make these observations the capsule of monomer-suspended iron crystals was polymerized by an appropriate means (159) to a solid block and this block was ultrathin sectioned for electron microscopy. The sections were cut at known directions to the direction of the field. This work is still continuing with improved technique and with higher magnetic fields. As a result of the work to date with low iron concentrations and relatively low fields a general "streaming" of the single crystalline rods and needles in the direction of the field has been observed. Micrographs have shown a tendency for the rods to attach end-to-end in a chain-like arrangement in a straight line or often in circles as the particles have sought to minimize their energies. Palisade configurations were sometimes seen but these were usually artifacts introduced by fortuitous cutting of a dendritic particle along and close to a primary stem.

The method is being further developed to provide a means of obtaining statistically quantitative estimates of the packing fraction for correlation with magnetic measurements on identical specimens, and to give direct observation for the first time of some of the theoretical aspects of fine particle magnetics, particularly shape anisotropy.

OTHER PHYSICAL PROPERTIES. The electron microscope studies were instrumental in explaining other properties of these crystals. The fine particle sizes and interstices in the microstructures explain the high specific surface of about 20 m^2/g. The minute nature of the crystallites also accounts at least partly for their extremely high surface energy. As a corollary to the highly crystalline nature of the particles, the high incidence of available crystal corners and edges, and the nature of the dendritic growth itself, in addition to surface energy there are "built in" strain energies due to lattice deformations. These characteristics impart such high energies that each crystal reacts chemically rather than as a particle of the element whose name it bears. The high energy associated with the monolithic, crystalline particles makes them extremely acitve physio-chemically so that they react readily and molecularly with metals, plastics and biochemical compounds to form new materials of unexpected physical properties.

MEDICAL APPLICATIONS. In addition to its fundamental interests in the electron microscopy of colloidal crystal systems, the Department of Physics has been interested in the alpha iron crystals because of projected medical investigations, suggested for them first by Michael W. Freeman. Armed with pertinent data* on their physical,

*The solid state properties of the material have been established and reported before several scientific groups for their criticism and study. The investigations have been reported before two conferences on Magnetics sponsored by the American Institute of Electrical Engineers, before the American Crystallographic Association, the Electron Microscope Society of America, and the Metal Powder Association.

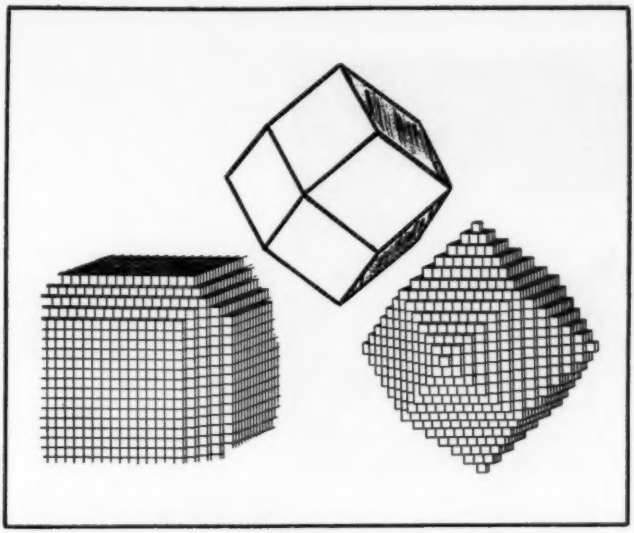

Figure 1

Drawings to demonstrate how a rhombic dodecahedron may be developed from the cube by regularly removing rows of cubelets. *A* shows the cube with rows of component cubelets being removed to develop the planes parallel to the faces of the dodecahedron. *B* shows the completed dodecahedron. *C* is a planar representation of *B*.

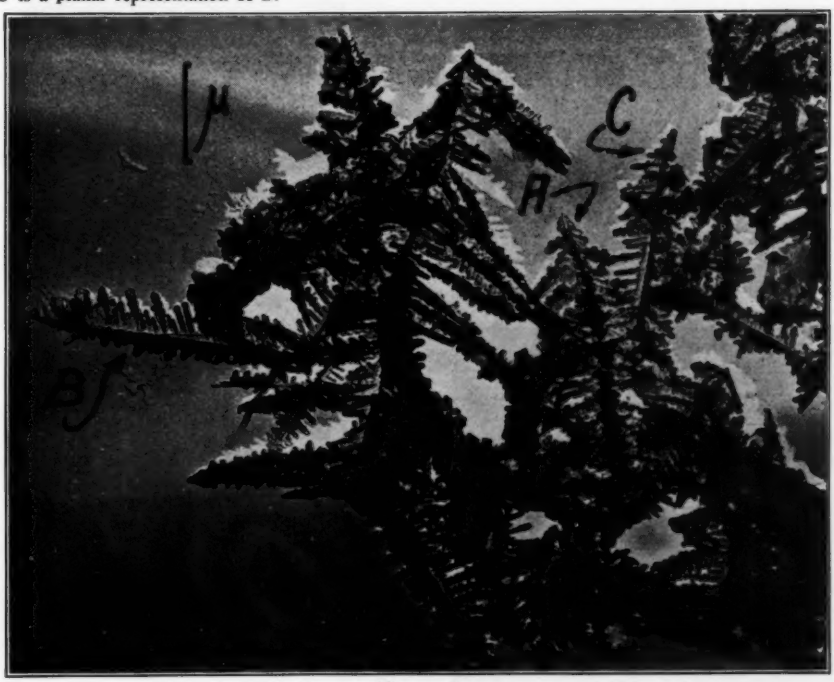

Figure 2

Electron micrograph, X15,000 of a carbon replica of strong alpha iron dendrites, partially etched with HCL. Note: in all micrographs the line represents a micron.

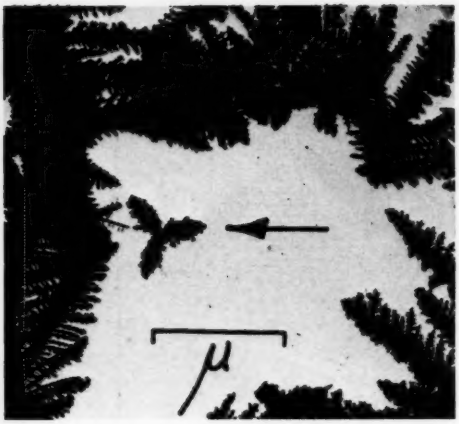

Figure 3

Electron micrograph, X20,000, of strong dendrites of alpha iron, showing at the arrow an example of a dendrite oriented 'end-on' so that three secondary branches at 120° can be seen.

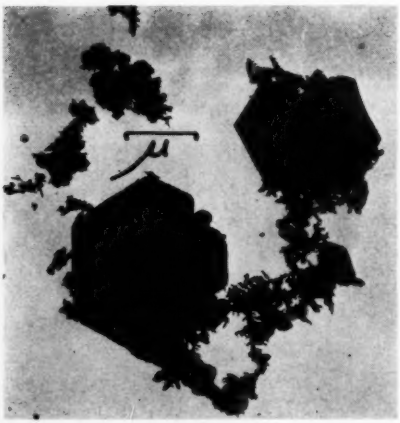

Figure 4

Electron micrograph X10,000 of platelet crystals of alpha iron.

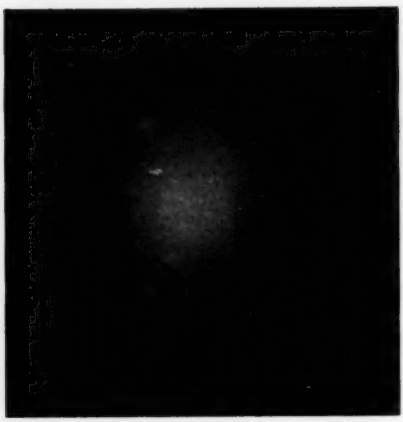

Figure 5

Selected area electron diffraction pattern of a single platelet crystal of alpha iron.

Figure 6
Electron micrograph, X19,000, of nodular rods and needles of alpha iron.

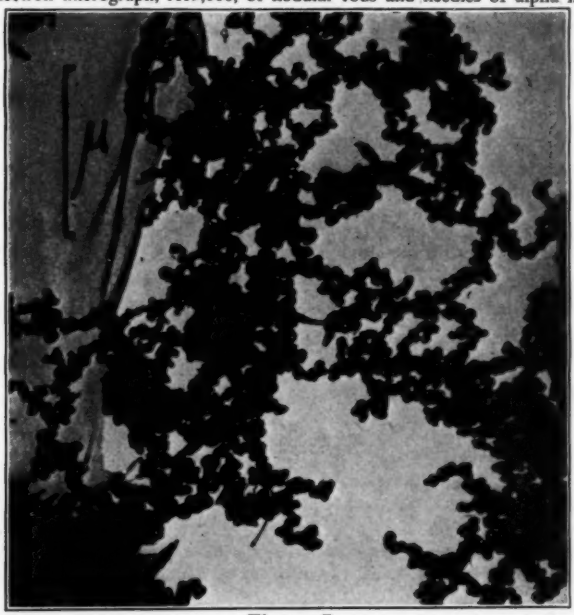

Figure 7
Electron micrograph X25,000 of chains of discrete particles of alpha iron. Average diameter of the sample 400 A.U.

magnetic and other properties one can evaluate the potential uses of the crystals for the medical field.

An immediate consideration is the employment of this knowledge in the treatment of cancer and in basic biochemical problems. A first emphasis is to develop a new method for the treatment of tumors with radioactive, magnetic iron† crystals concentrated in situ by external magnetic fields as prescribed. Many tumors are known to maintain a highly vascular functional system to sustain a preferred nutritional status with respect to surrounding healty tissue. Such tumors with good vascularity would be chosen for treatment at the outset. Electron microscope studies have shown that the crystals are producible with diameters as low as 400 and 200 A.U., Fig. 7, and with a variety of shapes, while still keeping them well within the limits necessary for circulation in the finest capillaries. It is known that the finest capillaries in the lungs, for example, are about 5 u in diameter. The volume of an average red blood cell would be equivalent to about 11 million individual 200 A.U. particles. If the iron crystals were made radioactive and still retained their ferromagnetic properties they could be used for radiation therapy of cancer tumors through the route of the circulatory system. Introduced into this system it is conceivable that their concentration, intensity, and exact localization could be controlled by external magnetic fields to pin-point radiation at the site of a neoplastic lesion with little or no destruction of healthy tissue. As a preliminary effort in this work electron microscopy of ultra-thin sections would be used along with histo-biochemical methods to determine the ultimate fate of the iron crystals. It is possible that by use of crystals of appropriate shape and size, radiation could be delivered to specific locations in the reticular endothelial systems, which are known to retain particles in specific ranges of size and shape* preferentially.

There will be many preliminary investigative steps in the program, each of which may, in its own development, contribute worthily to the sum of basic scientific knowledge. Among these will be problems related to proper preparation and introduction of the iron. The whole effect of radiation from the radioactive iron crystals upon various facets of the human body, especially on the reticular endothelial systems will have to be studied. A fascinating side of the physical problem will be the whole question of concentration of ferromagnetic particles in a living organism by external magnetic fields. It may be necessary to coat individual particles either to prevent physiological reactions with them, or to filter their radiation, the while maintaining their ferromagnetic properties.

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†The two radioactive isotopes of iron are Fe55 with a half life of 2.9 years, a gamma emitter only; and Fe59 with a half life of 46.3 days, both a gamma and beta ray emitter. The Fe55 is present in twice the abundance of the Fe59.

*"Effet de l'injection de saccharate d'oxyde de fer sur l'activite phagocytaire du systeme reticulo — endothelial chez le Rat". B. Benacerraf, C. Stiffel et G. Biozzi, *C. R. Seances Soc. Biol., 150,* 1161 (1956).

"Observations on the Reticulo — Endothelial Elements of the Liver and Spleen of the Rat Following Injections of Gelatin — Stabilized Gold Colloid". Arlene R. Seaman and Irwin M. Murray. *Acta haemat. 17,* 49 (1956).

The high physio-chemical activity of the particles is indicative of other biological applications. For example it is proposed to observe the effects of the iron crystals, in radioactive or non-radioactive form, in the study and treatment of leukemias. If a physiological preference can be demonstrated for hemoglobin or some enzymatic activity, the crystals may be used to reinstate normal hemopoesis*. Throughout all such studies a research by-product would continue to concern basic physiological phenomena not identified clearly heretofor.

The above sets forth some of the final objectives inherent in the medical uses of the submicron iron crystals and indicates the minimum essential preliminary steps.

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*"Intramuscularly Administered Iron in the Treatment of Iron-Deficiency Anemia". Albert B. Hagedorn, *J.A.M.A., 164,* 1642 (1957).

NUTRITION AND METABOLISM DIVISION
AND BIO-ORGANIC DIVISION

Left to right: Mr. William L. Anthony, Dr. Harold C. Choitz, Mr. James A. Edmondson, Dr. William T. Beher, Dr. Helen Lees, Dr. O. H. Gaebler, Miss Dorothy K. Reid, Dr. Paul D. Bartlett, Miss Shirley A. Shelata, Dr. Harvey F. Fisher, Mrs. Rachel L. Glovinsky, Mr. Thomas G. Maskaleris, Miss Dorothy A. Kurrie.

DEVELOPMENT OF THE BIOCHEMISTRY DEPARTMENT

O. H. GAEBLER

In 1947, when the Edsel B. Ford Institute for Medical Research was begun, the course to be followed in developing a Department of Biochemistry was self-evident. Nationally the shortage of trained personnel resulting from World War II was acute. Locally research facilities were in that contemplated stage which prospective staff members analyze with a dubious facial expression. On the other hand, the possibility of starting with graduate students, and building up personnel and facilities together, was wide open. Under an arrangement made in 1934, with the Chemistry Department of Wayne University (now Wayne State University), seven candidates for the M. S. degree with major in biochemistry had completed the research requirement for this degree in the writer's laboratory, and a doctorate program had been started in 1946. Establishment of certain lines of interest was, therefore, closely intertwined with the thesis topics and subsequent adventures of a series of graduate students.

The first predoctoral fellow in the Department was Paul D. Bartlett, who joined the staff as an associate on July 1, 1948, shortly after completing his doctorate. His thesis (6) and publications from it (11, 12) formed the background for a series of studies on the mechanism of nitrogen storage during induced growth (18, 21, 22, 34, 35, 57, 58). The second predoctoral fellow, William T. Beher, completed his studies for the doctorate in February 1950. His thesis (26) dealt with certain analytical and metabolic problems in the steroid field. Metabolic aspects of his interest in sterols and steroids have been continued in a series of studies of factors influencing absorption, deposition and mobilization of cholesterol (83, 88, 109, 135, 149), while analytical aspects have been developed in studies, carried out in collaboration with Jonathan Parsons of the Physics Department, in which the value and limitations of two methods — x-ray diffraction and infra-red absorption — are explored (106, 107, 108, 123, 153, 157).

Two other students have completed doctoral programs in the Department and embarked upon careers elsewhere. Stanley Ellis, who was graduated in 1951, has had a fruitful career in the laboratories of Herbert M. Evans and Alfred Wilhelmi. While his thesis was concerned with purine precursors (47), his subsequent interest has been in growth hormone and other pituitary factors. Alexander C. Zuchlewski participated in studies of the relationship between growth hormone and insulin (143), investigated effects of growth hormone on activities of glutamic acid dehydrogenase and transaminases (148), and, after completing his Ph. D. degree (90) in 1954, entered Wayne State University College of Medicine as a medical student.

Another line of investigation — enzyme chemistry as such — was begun by James C. Mathies, who joined the staff July 1, 1948, after completing his M. S. degree here and his doctorate work at the University of Washington, Seattle. During the first two years, he participated in an extensive departmental program in which effects of growth hormone on activity of tissue enzymes and coenzymes (15, 16, 19, 45, 51, 55, 63) were investigated. Meanwhile, he supervised construction of a laboratory suitable for enzyme chemistry, and when this was completed, carried out a series of

studies on purification and characterization of alkaline phosphatase (49, 79). Dr. Mathies is now at the Swedish Hospital, Seattle, Washington, and is participating in the development of research facilities there.

The laboratory which Dr. Mathies developed served as the starting unit for a greatly expanded program in enzyme chemistry, developed by Dr. Thomas P. Singer and Dr. Edna B. Kearney, who joined the staff October 1, 1954. The work on succinic dehydrogenase which this team began at the Enzyme Institute of the University of Wisconsin has been continued here, and expanded in collaboration with a series of postdoctoral fellows and visiting scientists. While certain aspects of the program may be described as "purification and characterization", this feature represents obstacles encountered and overcome, rather than the motivation or goal of the journey. Actually, the program is inspired by a lively interest in the mechanism of certain crucial steps in biological oxidation, the comparative biochemistry of the same process in mammals and bacteria, the role of metals, notably iron, in the action of certain enzymes, and many other fundamental problems.

Two organic chemists, Dr. W. M. Holliday and Dr. Milton Madoff, made important contributions in studies involving use of the stable isotopes carbon-13 and nitrogen-15. Dr. Holliday synthesized 3-acetylpyridine with C-13 in the carbonyl group. After feeding this compound, Dr. Beher isolated heavily labeled N-methyl nicotinamide from the urine, thus demonstrating conversion of 3-acetylpyridine, previously considered an antimetabolite for nicotnic acid, into a biologically active nicotinic acid derivative. It has since been demonstrated elsewhere that blacktongue will not develop in dogs if a suitable amount of 3-acetylpyridine is added to the diet at the time when nicotinic acid is removed. Dr. Madoff extended a program initiated by Dr. Bartlett, by synthesizing urea and various amino acids labeled with N-15. Studies in which these compounds have been, or are being, used are mentioned in another section.

Members of the Department have participated in organization and publication of two of the International Symposia (102, 125) in the series which was initiated by the Executive Director, Dr. Robin C. Buerki, in 1953. Some of our more recent studies on effects of growth hormone on nitrogen metabolism were presented in the "Growth Hormone Symposium" (100, 101). Earlier related studies had been summarized in a symposium elsewhere (85). The beginnings of the expanded program in enzyme chemistry were presented in a contribution (126) to the "Enzyme Symposium". International congresses in which staff members have participated include the Third International Congress of Biochemistry at Brussels, in 1955, and International Physiological Congresses at Montreal, in 1953, and at Brussels, in 1956. Before the ten-year period covered in this report ended, arrangements had been completed to participate in the International Congress on Clinical Chemistry in Stockholm, and in an International Symposium on Enzyme Chemistry, in Japan.

Other extramural activities include presentation of lectures and seminars, and participation in scientific organizations. While the writer was on the staff of the Department of Laboratories of Henry Ford Hospital, he was invited to initiate courses in general biochemistry and biochemical methods of analysis in the Chemistry

Department of Wayne University. This activity was continued after the Institute was begun, and eventually led to formation of a Biochemistry Division, with which Dr. P. D. Bartlett and Dr. W. T. Beher have continued to collaborate. The writer also gave an annual series of 12 lectures on intermediary metabolism, in the Department of Physiological Chemistry, from 1949 until 1955.

In 1954, Dr. W. T. Beher gave an invited lecture at North Central College, Naperville, Illinois. Dr. P. D. Bartlett has given seminars dealing with protein metabolism, at Wayne State University, and in the basic science series of Henry Ford Hospital.

Dr. Edna B. Kearney and Dr. T. P. Singer have given joint lectures dealing with the action of succinic dehydrogenase, and with metabolism of sulfur containing amino acids, at Western Reserve University, University of Wisconsin, Naval Medical Research Institute, and University of Michigan. Dr. Singer also presented a seminar at Michigan State University, and a lecture on "Experimental Foundations of the Concept of Metal-Flavoprotein Catalysis" in the Frontiers in Chemistry series of Wayne State University. Dr. Vincent Massey, on completion of a two-year postdoctoral fellowship in the Enzyme Division, presented lectures dealing with electron transport mechanisms and the role of metals in biological oxidations, at the universities of Minnesota, Utah, and California, and at Oak Ridge National Laboratories. Miss M. G. P. J. Warringa, a Fulbright fellow, also concluded the fellowship with a tour that included seminars on "Succinic Dehydrogenase Action in Obligate Anaerobes", given at seven major universities and national laboratories.

The writer was a member of the American Board of Clinical Chemistry for 6 years, serving as vice-president and as secretary-treasurer, and is currently vice-president of the American Association of Clinical Chemists.

Analytical work involved in studies of the Department of Pediatrics has been performed by assistants in the Biochemistry Department throughout the ten-year period, and transfer to new quarters has only recently been completed. In its initial stages, the Endocrinology Laboratory program was also housed with Biochemistry. In both these instances the programs were directed by the heads of the corresponding clinical departments; also in both instances, an assistant from the Biochemistry staff was transferred to the clinical department to facilitate continuity of projects in progress at the time of separation.

The goal which has been reached during the ten-year period since the Department was begun is the establishment of three active divisions: Nutrition and Metabolism, Bio-Organic Chemistry, and Enzyme Chemistry. Each division has a chief and a senior associate with complementary but independent programs. The three divisions have a strong common interest in metabolic processes.

STUDIES ON THE MECHANISM OF NITROGEN STORAGE

PAUL D. BARTLETT

One of the many interesting problems which have attracted investigators in the field of protein metabolism is the mechanism by which a rapidly growing immature organism retains nitrogen and accumulates body protein. For the greater part the author's research program has also been focused upon this problem, and has been guided by the thought that comparative study of nitrogen metabolism in animals in various states of the growth process might reveal certain distinct metabolic differences which would throw some light on the regulatory mechanisms involved. In this connection studies of the metabolic effects of growth hormone in animals in which the growth process has been arrested, either by hypophysectomy or by attainment of the adult state, have been particularly useful.

As is clearly indicated from the titles in the Publication List, the scope of attack on the problem has included studies of amino acids (18, 34), glutathione (134), and plasma protein metabolism (58); muscle, hepatic, and renal transaminase (34, 35): phosphate and pyruvate-activated glutaminase (21, 22); hepatic and renal dehydropeptidase I (52); rates of amino acid catabolism, protein synthesis and degradation; and the size of the nitrogen and urea pools (57, 69, 92, 100). These studies represent an attempt to characterize the growth process, and incidentally also the process of aging, in terms of nitrogen metabolism and enzyme systems involved in the metabolism of nitrogen compounds.

Quite early during the course of studies on the mechanism of action of growth hormone, observations were made by several groups of investigators suggesting that the hormone might function indirectly through regulation of fat metabolism. This is of special interest since accelerated fat catabolism would not only provide the energy requirements for the endergonic process of protein synthesis but also exert a protein-sparing effect during periods of metabolism when carbohydrate was not readily available as an energy source. In considering a number of mechanisms which might explain a regulatory effect of growth hormone on fat metabolism, we were particularly attracted to the hypothesis that control of the concentration of coenzyme A might be the mechanism involved. Initial studies of tissue coenzyme A concentrations, in both normal and induced states of growth, have resulted in the exciting finding of an apparently direct relationship between liver coenzyme A concentration and the growth process in normal rats (116). Currently these studies are being extended to include observations on the short-term effects of growth hormone on concentrations of oxidized and reduced forms of the cofactor in both normal adult and hypophysectomized rats. While it is too soon to adequately assess the significance of our data in relation to the possible effects on fat metabolism, it seems of special interest that in the relatively short period of 5 hours following stimulation with a single dose of growth hormone, in both normal adult and hypophysectomized rats, the balance of reactions requiring or producing liver coenzyme A appears to shift so that the reduced form is significantly increased. In 16 hours this effect has disappeared and the ratio of oxidized to reduced coenzyme A has returned to values similar to those observed in unstimulated rats.

Although indirect evidence appears to support the view that effects of growth hormone on nitrogen metabolism are mediated through alterations produced in fat metabolism, no unequivocal direct effects of the hormone on fatty acid catabolism have been demonstrated. Since the above hypothesis necessarily implies that fat is the preferential source of energy involved in the mechanism by which growth hormone induces nitrogen storage and accumulation of body protein, one might reasonably expect the metabolic pattern of handling a test load of amino acids administered under a condition in which fat is preferentially metabolized to differ from the pattern when carbohydrate metabolism is serving as the energy source. In order to test this supposition, we are presently engaged in a study of the effects of growth hormone on plasma total free alpha-amino acid nitrogen, glutamine nitrogen, and blood urea nitrogen, during the 3 hour period immediately following infusion of a test load of amino acids (10% Amigen) into adult dogs in which either fat or carbohydrate is being metabolized. In experiments in which the effects of fat are being studied, the fat is fed 3 hours prior to intravenous administration of the test load of Amigen. In experiments with carbohydrate, glucose is fed 1 hour prior to infusion of the Amigen.

An increasing interest in the bioenergetics of growth at the subcellular level of organization is shown by studies now in progress in this laboratory in which we are investigating the process of oxidative phosphorylation in liver mitochondria obtained from rats in various states of the growth process.

The opportunity to discuss basic research problems in conjunction with the clinical problems of the Henry Ford Hospital medical staff has proven particularly stimulating. One such discussion has recently emphasized the problems confronting the clinician in the nephrotic syndrome. In considering possible mechanisms which might explain the pathogenesis of glomerular changes and edema in the nephrotic syndrome, several observations suggest that a shift in the metabolism of trytophane toward the formation of 5-hydroxytryptamine might be an etiological factor. In both the rat and the dog, for example, 5-hydroxytryptamine produces an antidiuretic effect and a decrease in the glomerular filtration rate. The problem, one of mutual interest to the staff of the Pediatrics Clinic and the author, is currently being investigated.[1]

[1]This study is being supported in part by a Grant-in-Aid from the Michigan Chapter of the National Nephrosis Foundation, Inc.

ENZYME DIVISION

Left to right: Dr. Antonio Giuditta (Fulbright Fellow), Mr. Paul Bernath, Dr. Francisco Lara (Rockefeller Fellow), Dr. Vincent Massey, Dr. Edna B. Kearney, Dr. Thomas P. Singer, Mrs. Joanne Wilberding, Mrs. Lillian Cummings, Mr. Donell Thomas, Miss Maria Warringa (Fulbright Fellow), and Mr. Robert Vukmirovich.

ELECTRON TRANSPORT AND OTHER PROBLEMS

T. P. SINGER* AND EDNA KEARNEY

During the summer of 1954 the time seemed ripe to expand the activities of the Institute in the realm of enzyme chemistry and to initiate a systematic research and educational program in this field. In October 1954, under the aegis of the Biochemistry Department, the Enzyme Division was set up with the writers and Miss Norma Zastrow as its initial members.

The numerous problems involved in creating suitable facilities for the large scale isolation of enzymes, in instrumentation used in the study of their mode of action, and in providing trained personnel have been met with help from several sources. With generous support from institutional funds, an excellent laboratory for large scale isolation was added to the existing nucleus of laboratory space and equipment, and the unit was eqiupped to handle most problems encountered in the isolation, assay, and detailed investigation of enzymes. The Research Committee of the American Heart Association agreed to transfer the established investigatorship of Dr. Singer, and further provided a long-term grant-in-aid in support of the contemplated program. The National Heart Institute, U. S. Public Health Service, intent on expanding an evident interest in enzyme chemistry and intermediary metabolism in this area, has provided generous long-term support of research starting in October 1954. Further extramural support of the program has come from a contract between the Institute and the Office of Naval Research, U. S. Navy, involving research in the general area of biological oxidations. These research grants have provided funds for two post-doctoral fellowships, occasional visiting investigators, two research assistants, and for additional capital equipment and supplies.

The two fellowships have been held by Dr. Vincent Massey, who came here in October 1955 from the University of Cambridge, and Dr. Antonio Giuditta, of the Stazione Zoologica, Naples, Italy, who joined the group in 1956 upon the receipt of a Fulbright Travel Award. Since May, 1957 the position vacated by Miss Zastrow has been occupied by Dr. R. Ringler, a graduate of Michigan State University.

The scope of the activities of the group has been greatly increased as a result of collaboration with other scientists in the Detroit area and with visiting scientists who have worked in the Division for varying periods. In the first category mention should be made of a collaborative program with Dr. Caroline Hebb of the Detroit Institute of Cancer Research on the adaptive development of enzymes concerned with electron transport. Visiting scientists who have worked in the enzyme laboratory have included Professor Enzo Boeri, University of Ferrara, Ferrara, Italy; Dr. Francisco J. S. Lara, University of Sao Paulo, Ribeirao Preto, Brazil (Rockefeller Fellow); Miss M. G. P. J. Warringa of the Netherlands National Defense Research Council Laboratories, Rikswijk, Holland (Fulbright Fellow); and Mr. Oliver Smith, graduate student at the Department of Microbiology, Western Reserve University School of Medicine.

The two main activities of the enzyme group have been research and the training of specialists in enzyme chemistry.

*Established Investigator, American Heart Association.

Recognizing that successful solution of the major problems in the field of enzyme chemistry is greatly facilitated by the collaboration of investigators of varied training and background, the majority of the investigative work has represented the voluntary cooperation of several individuals in the group in the broad areas of biological oxidations and electron transport mechanisms. Reduced to its simplest terms, the aim of these efforts is to elucidate the chemical machinery whereby the energy residing in the chemical structure of foodstuffs is converted to a form which the living cell can utilize for its manifold activities. World-wide interest in this problem during the past three decades has resulted in a virtually complete mapping of the intricate pathways whereby individual sugar, fat, amino acid, and nucleic acid molecules are degraded to CO_2, water, and nitrogenous end products and are synthesized from the latter. Furthermore, the individual enzymes concerned with each step have been isolated and purified in many cases and obtained in crystalline or homogeneous form in others. While these accomplishments unquestionably represent a major triumph of modern biochemistry, some of the crucial problems involving the transformation of chemical energy during oxidations in the living cell remain quite obscure. Thus, while it has been known for almost 30 years that the oxidation of succinate to fumarate is a key step during the "terminal oxidative cycle" (Krebs cycle) of all foodstuffs, knowledge of *how* the energy of this chemical transformation is trapped in a utilizable form has been virtually nil. The oxidation involves the loss of a pair of hydrogen atoms and, therefore, of a pair of electrons, in the conversion of succinate to fumarate, and both pairs end up as water after union with O_2, the ultimate cellular oxidant. The union of hydrogen atoms with O_2 is not a direct one, but proceeds by an intricate pathway, involving many enzymes (including the so-called cytochrome system). The purpose of an intricate step-wise oxidation is to permit the gradual release of energy, in contrast to a wasteful, direct, explosive step of low efficiency. It is this "electron-transport" pathway and the enzymes that catalyze it that hold the secret of biological energy generation. It is to the study of this pathway and of its enzymes that the major efforts of the group have been devoted since its inception.

The succinic dehydrogenase system, referred to above, has not been unique as an area of metabolism which has defied the efforts of many able biochemists to unravel its mode of action. Rather, it is the prototype of the electron transport machinery and it may be safely predicted that its understanding will permit broad generalizations which may ultimately lead to the full understanding of the steps between the absorption of foodstuffs and the utilization of their chemical energy for muscular contraction, secretion, growth and other processes.

No account of a chemical transformation in a living system is complete, however, until the action of the enzyme, the catalytic agent which brings about the change, is explained on a molecular and electronic level. The elucidation of the mode of action of enzymes in this regard is in its infancy since knowledge of the structure of proteins, a prerequisite for a rational explanation of enzyme action, is all too scarce. Hopeful signs of progress have been made with some of the simpler hydrolytic and oxidative enzymes. An altogether different level of complexity is encountered in the enzymes of electron transport, which often have several non-protein "prosthetic

groups" (metals and vitamin derivatives) playing a role in the catalysis. Much of the current and future work of the group is aimed in this direction.

In difficult problems of this type the indirect route is sometimes the fastest. Thus, a comparison of the mode of action of homologous oxidative enzymes from many cells: mammalian tissues, plants, aerobic and anaerobic bacteria has served as a powerful tool in the elucidation of the role of the various components.

Lastly, attention may be called to the fact that this type of study has not only fundamental interest to the biologist intent on understanding the machinery of living processes but also some very practical implications. The "trace substance hypothesis", the working hypothesis of the majority of biochemists, holds that most or all substances capable of eliciting major biological effects in trace amounts (many drugs, poisons, hormones, toxins) act directly or indirectly by affecting enzymes. The succinic dehydrogenase system is a good example of an enzyme system which is profoundly affected *in vitro* and *in vivo* by a number of trace substances. The elucidation of the mode of action of these agents on the individual enzymes is an integral part of the effort of the group.

Another aspect of the program of the enzyme group involves the training of postdoctoral fellows and visiting scientists in both general enzyme chemistry and in specialized aspects thereof. This is accomplished partly by means of frequent research conferences, partly by a recently inaugurated departmental seminar program in which we have been fortunate in obtaining the participation of noted speakers from other institutions.

METABOLISM OF LIPIDS AND 3-ACETYLPYRIDINE

WILLIAM T. BEHER

Since completion of research for a doctoral thesis (26) in this Department, the writer, with Mr. William L. Anthony and Mrs. Gizella Baker as collaborators, has engaged in 3 principal lines of investigation which are briefly discussed below.

1. Metabolism of 3-Acetylpyridine

Reference has already been made (page 122) to studies in which labeling with carbon-13 was employed to demonstrate *in vivo* conversion of 3-acetylpyridine to a labeled nicotinic acid derivative. The question arose whether during the metabolism of 3 A.C.P. to N^1-methyl nicotinamide, the methyl group of 3 A.C.P. was transmethylated to suitable acceptors or oxidized. To answer this question, a new and highly successful organic synthetic method of labeling the methyl group with C-14 was developed. When the resulting compound was administered to rats and the expired CO_2 collected, about 20% of the injected activity could be accounted for in the first 8 hours after injection — the bulk of it in the first 6 hours — indicating a rapid oxidation. While a considerable amount of activity was excreted in the urine (40% of injected activity in 24 hours), none was detected in the feces. Urinary C-14 activity was not present as HCO_3 or unaltered 3 A.C.P., but only as a urinary product which has not yet been isolated. This substance seems to be an amino acid or other amphoteric compound.

In a series of *in vitro* studies, the tissues responsible for the oxidation of 3 A.C.P. are being investigated. Tissue slices of liver, kidney, muscle, and spleen are incubated in Ringer's solution containing C-14 3 A.C.P. The CO_2 produced is collected and assayed for C-14 activity. Preliminary results indicate active metabolism in the liver and kidney with little or none in the spleen or muscle. Because of the number of probable steps necessary for oxidation, combination studies (kidney + liver) are contemplated. It is hoped that these studies will eventually lead to an understanding of the mechanism of single methyl oxidation.

Studies on the effects of 3 A.C.P. on enzyme systems requiring Co I and II have been completed. If the substitution antimetabolite theory applied in these cases, one would expect 3 A.C.P. to inhibit these reactions. However, in a study of glucolysis by R B C's, this did not occur even with 1% of 3 A.C.P. Moreover, synthesis rates of cholesterol and lipids in liver slices, as indicated by C-14 uptake, were retarded only at high 3 A.C.P. concentrations. It thus seems that 3 A.C.P. does not function by substitution in DPN or TPN (at least in the systems studied).

3 A.C.P. is extremely toxic in rats at the 15 mg per day dose level, but this toxicity can be reversed by prior (but not simultaneous) administration of nicotinamide. Theoretically this might be explained by a buildup of enzyme systems containing DPN or TPN which oxidize 3 A.C.P. to niacin and its derivatives. Experiments on oxidation rates of C-14 (methyl) labeled 3 A.C.P. indicate a slightly increased rate of oxidation of 3 A.C.P. in the animals pretreated with nicotinamide.

2. Studies of Lipid Metabolism

a. *Experimental Atherosclerosis.*

It is known that experimental atherosclerosis can be produced with varying degrees of ease in different species. For example, while dietary cholesterol alone is sufficient in the case of the normal chick and rabbit, in the case of dogs and rats this substance will produce hypercholesterolemia and atherosclerosis only in the presence of hypothyroidism. Therefore, it seems possible that hypophysectomized rats, which due to the absence of the thyrotrophic activity lack the thyroid hormone, might be especially susceptible to experimental atherosclerosis. Since such animals are also deficient in many other hormones such as adrenal corticotrophic hormone, growth hormone, and the gonadotrophic hormones, the effects of these substances on the production and regression of experimental atherosclerosis can also be studied.

Studies previously carried out (135, 149) have shown that the extremely slow turnover rate of cholesterol and other lipids in the rabbit aorta, make this species unsatisfactory for the study of regression of aortic plaques. In some cases actual increases in plaque area and total lipids were observed during the regression period. These increases were probably due to the deposition of excess blood lipids. Therefore, while it is possible that satisfactory studies might be undertaken if sufficient time were allowed to clear the excess blood and liver lipid, the turnover rate would probably remain much too slow for satisfactory results. A literature survey indicates that the chick might prove a satisfactory species for the study of aortic plaque regression, because of the higher turnover rate of lipids in its aorta. While the above outlined experiments on hypophysectomized rats should prove very valuable in the study of hormonal effects, we believe that the chick will be much more satisfactory in studying aorta plaque regression as influenced by such substances as β-sitosterol, choline, and surface active agents.

b. *Cholesterol Metabolism*

Perhaps one of the most important groups of compounds concerned with the endogenous metabolism of cholesterol, are the bile acids. These substances are not only of major importance in the primary absorption and intestinal hepatic recycling of cholesterol, but also appear to have a definite influence on the rate of mobilization, metabolism and tissue distribution of cholesterol. For example, we have shown (83) that bile acids added to a lipid-free diet, supplemented with cholesterol, increase the cholesterol deposition in mice livers. In other experiments not yet reported, dietary cholic acid retarded the mobilization of accumulated liver cholesterol in the mouse. These phenomena may be interdependent or independent; that is, cholic acid may act on cholesterol in either of two ways: (a) it may increase the absorption rate; or (b) it may decrease the degradation rate. To investigate these problems, three experiments will be carried out:

1. Absorption of cholesterol in the presence of varying amounts of cholic acid in mice.

2. The influence of cholic acid on the rate of cholesterol metabolism in the normal rat.

3. The influence of cholic acid on the rate of cholesterol catabolism in rats with elevated liver cholesterol.

c. *Studies of Substances which Inhibit Cholesterol Synthesis and Absorption.*

Since high cholesterol levels are associated with atherosclerosis, substances which lower serum and tissue cholesterol are being sought and investigated. In this laboratory (83, 109, 135, 149) both β-sitosterol and dihydrocholesterol were found effective in lowering serum and liver cholesterol. However, dihydrocholesterol, in addition, showed toxic effects. The extent and severity of these are currently being investigated in a joint project with the Pathology Department of Henry Ford Hospital.

In another approach to this problem, an effort is being made to find a substance which will inhibit the synthesis of cholesterol in an organism. Since the intermediates in the synthesis of cholesterol have recently been discovered, we wish to synthesize several derivatives of these and test their activity as antimetabolites.

3. Analytical Studies Using Infrared Spectroscopy

These are discussed in the article by Jonathan Parsons (Page 99) with whom the writer collaborated.

METABOLIC EFFECTS OF GROWTH HORMONE, CORTICOTROPIN, AND VARIOUS STEROIDS

OLIVER H. GAEBLER

During 1954 and 1955, the writer participated in arrangements for two International Symposia (102, 125). Research activity took the form of exploratory experiments which provided the basis for resumption of a more active program on completion of these assignments. Mr. Trieste Vitti, Mrs. Rachel Glovinsky, and Dr. Helen Lees, collaborated in developing such a program. Generous support for its inception has been provided by the Michigan Chapter, Arthritis and Rheumatism Foundation, and, for its expansion, by the Division of Research Grants, National Institutes of Health. Several lines of investigation, all requiring similar experimental procedures and instrumentation, are under way.

1. *Metabolism of Nitrogen-15 from Various Sources, During Induced Nitrogen Storage or Loss.*

Earlier studies, briefly summarized at a symposium (85), acquainted us with changes in the nitrogen balance and nitrogen partition which growth hormone elicits. Curiosity arose as to whether induced storage of nitrogen is general, involving nitrogen from all amino acids, or whether it has selective characteristics. Even if the name "growth hormone" is valid, present knowledge of transfer of amino groups makes it unsafe to assume that the nitrogen required for growth necessarily comes from each of the amino acids in the proportion that average amino acid composition of total body protein dictates. A less orthodox arrangement, though introducing an element of complexity, might supply one of control. Moreover, studies on changes in transaminase activity, initiated by Bartlett (34, 35) and extended by Zuchlewski (90), suggested the desirability of studying the metabolism of N-15 originating from ammonia, alanine, glutamic acid, and aspartic acid. Some observations on ammonia output in normal dogs receiving growth hormone, and in depancreatized ones receiving growth hormone with or without additional insulin, indicated that effects on ammonia formation, as well as on transamination, might profitably be studied by this approach. These experiments also include observations on incorporation of nitrogen-15 from the various sources into fibrinogen, during nitrogen storage induced with growth hormone, and during nitrogen loss induced with corticotropin. Anatomical distribution of the N-15 is also being studied.

2. *Characteristics of the Action of Corticotropin and Various Steroids on Nitrogen and Water Balances*

When growth hormone is injected into normal adult bitches, there is an increase in water intake that exceeds the rise in urine volume. An overnight increase in weight occurs; nitrogen storage begins at once and reaches a maximum in a few days. The entire process reverses quickly after cessation of therapy. After corticotropin (20 units daily), water intake rises less than urine volume, marked losses of weight and nitrogen occur, and, while the losses stop soon after cessation of therapy, restoration

of initial weight may require weeks, or may not occur until the diet is increased. To date, simultaneous observations have not been made on metabolic rate in these experiments. Effects of hydrocortisone acetate (15 mg. daily, intramuscularly), prednisone (10 mg. daily per os), and hydrocortisone ointment applied to affected areas in a metabolism dog which occasionally developed dermatitis, were similar.

3. *Certain Interrelationships of Pituitary, Pancreas and Adrenals*

It has long been established that adrenalectomy, in depancreatized cats and dogs, minimizes the accentuation of diabetes by growth hormone. We, therefore, attempted to reproduce these untoward effects by administering corticotropin or hydrocortisone to a fed depancreatized dog receiving a constant dose of insulin. Corticotropin (20 units daily), caused slight nitrogen loss but no increase in glucosuria. Hydrocortisone (15 mg. daily) caused neither nitrogen loss nor glucosuria, although the same dose, in normal dogs, caused marked nitrogen loss. Hydrocortisone (30 mg. daily) increased output of nitrogen, but not of glucose, which was drastically affected by growth hormone under the same dietary conditions. When the diet was altered by addition of 50 gm. of sucrose daily, some increase in glucosuria followed administration of corticotropin or hydrocortisone, but the situation was not alarming, as in experiments with growth hormone. Since the smaller dose of hydrocortisone caused marked nitrogen loss in normal dogs, the intriguing, if remote possibility, that insulin is involved in mobilization, as well as in storage of nitrogen, will be studied later.

4. *Toxicity of Growth Hormone*

In depancreatized dogs receiving constant amounts of food and insulin, small daily doses of growth hormone appear to be more dangerous than large single ones. We lost one animal during the second experiment, and considered anaphylaxis, but a second one was lost during the very first course of treatment. Other depancreatized dogs, like the normal ones, tolerate a series of 6 daily doses of 2.5 to 5 mg. of growth hormone without responses that are inconsistent with metabolic studies. Neither insulin nor hydrocortisone is of much avail when the situation has become dangerous to life. While we are in the descriptive stage of this induced upset, studies of its nature and possible prevention or reversal will be continud.

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